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Abstract: Adenoviruses (AdVs) are prevalent and give rise to chronic and recurrent disease. The human AdV (HAdV) species B and C, such as HAdV-C2, C5 and B14, cause respiratory disease, and constitute a health threat for immuno-compromised individuals. HAdV-Cs are well known for lysing cells, owing to the E3 CR1- encoded adenovirus death protein (ADP). We previously reported a high-throughput image-based screening frame-work and identified an inhibitor of HAdV-C2 multi-round infection, Nelfinavir mesylate. Nelfinavir is the active ingredient of Viracept, an FDA-approved inhibitor of the human immuno-deficiency virus (HIV) aspartyl protease, and used to treat acquired immuno-deficiency syndrome (AIDS). It is not effective against single round HAdV infections. Here, we show that Nelfinavir inhibits the lytic cell-free transmission of HAdV, indicated by the suppression of comet-shaped infection foci in cell culture. Comet-shaped foci occur upon convection-based trans-mission of cell-free viral particles from an infected cell to neighbouring uninfected cells. HAdV lacking ADP was insensitive to Nelfinavir, but gave rise to comet-shaped foci indicating that ADP enhances but is not required for cell lysis. This was supported by the notion that HAdV-B14 and B14p1 lacking ADP were highly sensitive to Nelfinavir, although HAdV-A31, B3, B7, B11, B16, B21, D8, D30 or D37 were less sensitive. Conspicuously, Nelfinavir uncovered slow-growing round-shaped HAdV-C2 foci, independent of neutralizing antibodies in the medium, indicative of non-lytic cell-to-cell transmission. Our study demonstrates the repurposing potential of Nelfinavir with post-exposure efficacy against different HAdVs, and describes an alternative non-lytic cell-to-cell transmission mode of HAdV.

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The FDA-approved drug Nelfinavir inhibits lytic cell-free, but not cell-associated non-lytic transmission of human adenovirus

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Abstract

Adenoviruses (AdVs) are prevalent and give rise to chronic and recurrent disease. The human AdV (HAdV) species B and C, such as HAdV-C2, C5 and B14, cause respiratory disease, and constitute a health threat for immuno-compromised individuals. HAdV-Cs are well known for lysing cells, owing to the E3 CR1- β -encoded adenovirus death protein (ADP). We previously reported a high-throughput image-based screening framework and identified an inhibitor of HAdV-C2 multi-round infection, Nelfinavir mesylate. Nelfinavir is the active ingredient of Viracept, an FDA-approved inhibitor of the human immunodeficiency virus (HIV) aspartyl protease, and used to treat acquired immunodeficiency syndrome (AIDS). It is not effective against single round HAdV infections. Here, we show that Nelfinavir inhibits the lytic cell-free transmission of HAdV, indicated by the suppression of comet-shaped infection foci in cell culture. Comet-shaped foci occur upon convection-based transmission of cell-free viral particles from an infected cell to neighbouring uninfected cells. HAdV lacking ADP was insensitive to Nelfinavir, but gave rise to comet-shaped foci indicating that ADP enhances but is not required for cell lysis. This was supported by the notion that HAdV-B14 and B14p1 lacking ADP were highly sensitive to Nelfinavir, although HAdV-A31, B3, B7, B11, B16, B21, D8, D30 or D37 were less sensitive. Conspicuously, Nelfinavir uncovered slow-growing round-shaped HAdV-C2 foci, independent of neutralizing antibodies in the medium, indicative of non-lytic cell-to-cell transmission. Our study demonstrates the repurposing potential of Nelfinavir with post-exposure efficacy against different HAdVs, and describes an alternative non-lytic cell-to-cell transmission mode of HAdV.

40 Introduction

41

42 Adenovirus (AdV) was first described in 1953 by Rowe and co-workers as a cytopathologic
43 agent isolated from human adenoids (1). More than 100 human AdV (HAdV) genotypes have
44 since been characterized by molecular genetics or serology and grouped into seven species (2,
45 3). HAdV species A, F and G replicate in the gastrointestinal tract, B, C and E in the respiratory
46 organs, and B and D in conjunctival cells of the eyes. Species B members have a broad
47 tropism, including kidney and cells of the hematopoietic lineage (4–6). HAdV-caused illness can
48 range from asymptomatic to lethal, especially in immunocompromised individuals (7–9). HAdV
49 outbreaks are frequent in military training camps, but also nursing homes, as recorded in
50 recurrent outbreaks of HAdV-E4 and HAdV-B7 (5, 10–13). To counter the disease burden, an
51 oral HAdV-E4/B7 vaccine was reintroduced, leading to a sharp decline in adenoviral disease
52 among military recruits (5, 14, 15). In addition to recurrent HAdV outbreaks, novel HAdV
53 variants emerge, some of them causing pneumonia and death of elderly with chronic diseases.
54 One of these emerging HAdVs is the HAdV-B14 variant 14p1, also known as 14a (16–20).
55 Furthermore, AdVs have a potential for zoonotic transmission (21). Cross-species infections to
56 humans from either non-human primates or psittacine birds have been reported from the USA
57 and China, respectively (22, 23). Despite the high prevalence (5, 24–26) and the broad use of
58 AdV as gene therapy vectors (27) as well as oncolytic viruses (28, 29) no FDA-approved
59 specific anti-HAdV treatment is available to date. Clinically, HAdV infections are treated with
60 Ribavirin, Cidofovir, or more recently, Brincidofovir, which all inhibit viral DNA replication (30,
61 31).

62

63 HAdV particles have been well characterized. They have a double-stranded DNA genome of
64 ~36 kilo base pairs (kbp) packaged into an icosahedral capsid of about 90 nm in diameter (32–
65 35). HAdV-C2 and C5 replication cycle has been extensively studied including entry, uncoating,
66 replication, assembly and egress from the infected cell (36–50). HAdV-C infects cells by binding
67 to the coxsackievirus adenovirus receptor (CAR) and integrin co-receptors, followed by
68 receptor-mediated endocytosis, endosomal lysis and microtubule-motor driven transport to the
69 nucleus, where it uncoats DNA and delivers the DNA into the nucleus (38, 51–62). The first viral
70 protein expressed is E1A, a multifunctional intrinsically disordered protein controlling the
71 transcriptional activity of all AdVs, as well as many cellular promoters, thereby affecting the cell
72 cycle, differentiation, transformation and apoptosis (63–68). Viral early proteins besides E1A
73 mediate immune escape, block activation of pro-apoptotic pathways and form nuclear viral DNA
74 replication compartments. Late viral proteins give rise to mature progeny virions upon limited
75 proteolysis of capsid proteins by the viral cysteine protease L3/p23 (69–71). Mature HAdV
76 progeny is released upon rupture of the nuclear envelope and plasma membrane, which
77 facilitates rapid viral dissemination and plaque formation *in vitro* (72–74). The convection forces
78 in the medium give rise to comet-shaped infection foci in cell cultures (72). Foci of infected cells
79 are also found in tissue, such as rat liver upon intravenous inoculation of HAdV-C5 (75).
80 Accordingly, acute HAdV infections trigger an inflammatory response, as shown in airways or
81 conjunctiva of susceptible animals (2, 76). In contrast to lytic virus transmission, direct cell-to-
82 cell transmission leads to round plaques, as shown with vaccinia virus (77–80).

83

84 The mechanisms of virus transmission are highly virus-specific. They comprise non-lytic
85 pathways involving the secretory-endocytic circuits, multi-vesicular or autophagic membrane
86 processes, cellular protrusions, or transient breaches of membrane integrity (80–84). In
87 contrast, lytic egress pathways further involve the destabilization of cellular membranes by viral
88 and host factors, often tuned by the cytoskeleton (37, 85–88). HAdV-C2 controls lytic cell death
89 by the adenovirus death protein (ADP), also known as 11.6K, as concluded from genetic and
90 overexpression studies (73, 74). ADP is a type III membrane protein transcribed from the CR1- β
91 region in the immuno-regulatory E3a locus. All HAdV-C members harbour homologous E3a
92 CR1- β sequences (e.g. 10.5K in HAdV-C5). Other HAdV species differ in their E3 region,
93 however (89–91). The N-terminus of ADP is lumenal and the C-terminus protrudes into the
94 cytosol (92). Following post-translational modifications, ADP is transported to the inner nuclear
95 membrane, where the N-terminus is intruding into the nucleus (93). At late stages, when capsid
96 assembly in the nucleus has commenced ADP expression is boosted (94, 95). The mechanism
97 of host cell lysis is still unknown, although necrosis-like, autophagic and caspase activities have
98 been implicated (96–99).

99

100 Here, we report that Nelfinavir mesylate (Nelfinavir in short) is an effective inhibitor of HAdV lytic
101 egress. The identification process of Nelfinavir is described in an accompanying paper using an
102 imaging-based, high content screen of the Prestwick Chemical Library (PCL) comprising 1,280
103 mostly clinical or preclinical compounds (100, 101). Nelfinavir is the off-patent active
104 pharmaceutical ingredient of Viracept, FDA-approved, which inhibits the human immuno-
105 deficiency virus (HIV) protease (102). The work here documents the repurposing potential of
106 Nelfinavir, which is effective against a spectrum of HAdV types in a post exposure manner.
107 Nelfinavir is partly, but not exclusively, active against ADP-encoding HAdV types, and uncovers
108 the appearance of round-shaped plaques, which arise upon non-lytic cell-to-cell viral
109 transmission.

Materials and Methods

Viruses

HAdV-C2-dE3B-GFP was previously described (72) (GenBank accession number MT277585). The virus was generated by exchange of the viral E3b genome region with a reporter cassette harbouring the enhanced green fluorescent protein (GFP) under a constitutively active cytomegalovirus (CMV) promoter. It was grown in A549 cells and purified by double CsCl gradient centrifugation (103). Aliquots supplemented with 10% (v / v) glycerol were stored at -80°C. HAdV-C2-dE3B-GFP was found to be homogeneous by SDS-PAGE and negative-stain analyses in transmission electron microscopy (EM). Recombinant HAdV-C2-dE3B-GFP-dADP was generated using homologous recombination according to the Warming recombineering protocols (104, 105). For a detailed protocol, see Supplementary methods. HAdV-C2-dE3B-GFP-dADP was plaque-purified and amplified, followed by two rounds of CsCl purification (106). Aliquots containing 10% (v / v) glycerol were stored at -80°C. HAdV-C2-dE3B-GFP-dADP was found to be homogeneous in SDS-PAGE and negative-stain analyses by transmission EM. Lack of ADP expression was confirmed by Western immunostaining using the rabbit α -HAdV-C2-ADP₇₈₋₉₃ antibody, obtained from William Wold and Ann Tollefson (Saint-Louis University, Saint-Louis, USA) (107).

HAdV types A31, B7, B11, B14a, B16, B34, C1, C6, D8, D30 and D37 were kindly provided by the late Thomas Adrian (Hannover Medical School, Germany) and were verified by DNA restriction analysis (108, 109). HAdV types B14 (19, 20) and B21a, isolate LRTI-6 (110) were kindly provided by Albert Heim (Hannover Medical School, Germany). HAdV-B3-pIX-FS2A-GFP and B35-pIX-FS2A-GFP contain an enhanced GFP open reading frame (ORF) genetically fused to the downstream end of the HAdV pIX gene using an autocleavage FS2A sequence (111–113). rec700 (114) and dl712 (115) were obtained from William Wold (Saint-Louis University, Saint-Louis, USA). rec700 is a recombinant HAdV-C5 containing C2 sequences from nucleotide -236 to 2437 of the E3 transcription unit, and comprises the C2 E3a ORFs 12.5K, 6.7K, 19K and ADP, as well as major parts of the E3b ORF RID α (10.4K protein) (116). Mouse adenovirus (MAdV)-1-pIX-FS2A-GFP and MAdV-3-pIX-FS2A-GFP were constructed as described (117, 118). HAdV-C2 and C5 were obtained from Maarit Suomalainen (University of Zurich, Switzerland). HSV-1-CMV-GFP is a recombinant HSV-1 strain SC16 containing a CMV enhancer/promoter-driven enhanced GFP expression cassette in the US5 (gJ) locus (119) and was kindly provided by Cornel Fraefel (University of Zurich, Switzerland). HSV-1-CMV-GFP was propagated in Vero cells and purified by sucrose sedimentation as described in (120, 121). All viruses were stored in small aliquots containing 10% (v / v) glycerol at -80°C.

Cell lines

A549 (human adenocarcinomic alveolar basal epithelium, CCL-185), HeLa (human epithelial cervix carcinoma, CCL-2) and HBEC (HBEC3-KT, normal human bronchial epithelium, CRL-4051) cells were obtained from the American Type Culture Collection (ATCC, Manassas, USA). HCE (normal human corneal epithelium) cells were obtained from Karl Matter (University College London, UK). CMT93 (mouse rectum carcinoma) cells were obtained from Susan

153 Compton, Yale School of Medicine, USA. A549, HeLa, HCE and CMT-93 cell cultures were
154 maintained in high glucose DMEM (Thermo Fisher Scientific, Waltham, USA) containing 7.5% (v
155 / v) FCS (Invitrogen, Carlsbad, USA), 1% (v / v) L-glutamine (Sigma-Aldrich, St. Louis, USA)
156 and 1% (v / v) penicillin streptomycin (Sigma-Aldrich, St. Louis, USA) and subcultured following
157 phosphate-buffered saline (PBS) washing and trypsinisation (Trypsin-EDTA, Sigma-Aldrich, St.
158 Louis, USA) bi-weekly. HBEC cells were maintained in endothelial-basal medium (ATCC,
159 Manassas, USA) and passaged 1:1 weekly following PBS washing and trypsinisation. Cell
160 cultures were grown at standard conditions (37°C, 5% CO₂, 95% humidity) and passage number
161 was limited to 20. Respective supplemented medium is referred to as supplemented medium.

162

163 **Compounds**

164 Nelfinavir mesylate (CAS number 159989-65-8) powder was obtained from MedChemExpress
165 LLC (Monmouth Junction, USA and Selleck Chemicals, Houston, USA). Compound was
166 dissolved in DMSO (Sigma-Aldrich, St. Louis, USA) at 100 mM and kept at -80°C or -20°C for
167 long-term or working storage, respectively.

168

169 **Cellular impedance measurement**

170 Impedance-based assays were performed using the xCELLigence system (Roche Applied
171 Science and ACEA Biosciences) as described previously (122, 123) according to the
172 manufacturer's instructions (124) in cell culture environment (37°C, 5% CO₂, 95% humidity) in
173 duplicates. The 16-well E plates have a gold-plated sensor array embedded in their glass
174 bottom by which the electrical impedance across each well bottom is measured. The impedance
175 per well, termed cell index (CI), is recorded as a dimensionless quantity. The background CI
176 was assessed following the addition of 50 µl supplemented medium to each well and
177 equilibration in the incubation environment. After 30 min equilibration, 9,000 A549 ATCC cells in
178 50 µl supplemented medium were added per well and measurement was started.

179

180 For the quantification of Nelfinavir toxicity, 50 µl of supernatant were removed 18 h later and
181 replaced by 2-fold concentrated Nelfinavir or DMSO solvent as the control dilution in
182 supplemented medium (final Nelfinavir concentration 0.4-100 µM in 100 µl / well). The control
183 was supplemented medium. Impedance was recorded every 15 min over 5 days. TC₅₀ indicates
184 the concentration of Nelfinavir, which caused 50% impedance reduction compared to the
185 solvent-treated cells. TC₅₀ was calculated by non-linear regression of solvent-normalized CI
186 over the concentration of Nelfinavir.

187

188 For the quantification of Nelfinavir effects on the cytopathogenicity of HAdV-C2-dE3B-GFP
189 compared to HAdV-C2-dE3B-GFP-dADP infection, 50 µl supernatant were removed 18 h later
190 and replaced with Nelfinavir- and virus-supplemented medium. 25 µl of a 4-fold concentrated
191 Nelfinavir (final concentration 0.4-100 µM) or corresponding DMSO solvent control dilution (final
192 concentration 1%) in supplemented medium or supplemented medium only were added to 50 µl
193 medium containing cells. Additionally, 25 µl of a 4-fold concentrated virus stock dilution were
194 added (final inoculum 1.68*10⁶ viral particle(s) (VP) / well HAdV-C2-dE3B-GFP and 2.68*10⁶
195 VP/ well HAdV-C2-dE3B-dADP, corresponding to ~30 plaque forming unit(s) (pfu) / well. The

196 delay of infection-induced cytotoxicity was calculated as time point at which the CI of the
197 infected cells had decreased by 50% relative to its maximum. Data analysis was performed
198 using GraphPad (GraphPad Software, Inc, version 8.1.2), and curve fitting was performed using
199 three-parameter [inhibitor] vs. response nonlinear regression.

200

201 **Fluorescence-based plaque forming assay**

202 Per 96-well, 15,000 A549, 10,000 HeLa ATCC, 30,000 HBEC, 30,000 HCE or 30,000 CMT-93
203 cells were seeded in 100 μ l of the respective supplemented medium and allowed to settle for 1
204 h at room temperature (RT) prior to cell culture incubation at 37°C, 5% CO₂, 95% humidity. The
205 following day, the medium was replaced by 50 μ l of the respective virus stock dilution giving rise
206 to 5 to 50 plaques per 96-well. 50 μ l Nelfinavir to obtain 0.1 to 50 μ M final concentration or
207 DMSO solvent control was also added, both in supplemented medium. For each experiment, a
208 non-infected, treated control was performed. For uphill plaque assays, medium volume was
209 increased to 150 μ l with identical virus and drug concentrations. For wash-in / wash-out
210 experiments, virus was incubated on the cells in supplemented medium for 1 h at 37°C, cells
211 were washed with PBS and 100 μ l drug dilution in supplemented medium was added. All
212 experiments were performed in four technical replicates or as indicated. Cells were incubated at
213 standard cell culture conditions. At the indicated time post infection (pi), the cells were fixed and
214 the nuclei stained for 1 h at RT by addition of 33 μ l 16% (w / v) para-formaldehyde (PFA) and 4
215 μ g / ml Hoechst 33342 (Sigma-Aldrich, St. Louis, USA) in PBS. Cells were washed three times
216 with PBS and stored in PBS supplemented with 0.02% N₃ for infections with viruses harbouring
217 a GFP transgene. For wild type (wt) viruses, cells were quenched in PBS supplemented with 50
218 mM NH₄Cl, permeabilized using 0.2% (v / v) Triton-X100 in PBS and blocked with 0.5% (w / v)
219 BSA in PBS. Cells were incubated with 381.7 ng / ml mouse α -HAdV hexon protein antibody
220 (Mab8052, Sigma-Aldrich, St. Louis, USA) and subsequently stained using 2 μ g / ml goat α -
221 mouse-AlexaFluor594 (A21203 or A32742, Thermo Fisher Scientific, Waltham, USA). Plates
222 were imaged on either an IXM-XL or IXM-C automated high-throughput fluorescence micro-
223 scope (Molecular Devices, San Jose, USA) using a 4x objective at widefield mode. Hoechst
224 staining was recorded in DAPI channel, FITC / GFP channel for viral GFP and TRITC / Texas
225 red channel for immunofluorescence hexon staining.

226

227 **Therapeutic index measurement**

228 The infection phenotype for each well was quantified using Plaque2.0 (101). The number of
229 plaques was determined based on the infection signal (viral GFP or hexon immunofluorescence
230 staining). Nuclei stained with Hoechst were segmented by CellProfiler (125). Infected nuclei
231 were classified based on the median infection signal per nucleus in CellProfiler. Data were
232 plotted and EC₅₀ (infected and treated cells), TC₅₀ (non-infected, treated cells), as well as the
233 corresponding standard error (SE) determined using curve fitting in GraphPad (GraphPad
234 Software, Inc, version 8.1.2) using three-parameter [inhibitor] vs. response nonlinear regression.
235 Mean TI₅₀ was calculated as EC₅₀ / TC₅₀ ratio of the means. The TI₅₀ SE was calculated by error
236 propagation.

237

238 **Quantification of viral protein expression**

239 Infection, HAdV hexon immunofluorescence staining, and imaging were performed in technical
240 quadruplicates, as described under Microscopic plaque assay. Single nuclei (Hoechst) were
241 segmented using CellProfiler (125). Median GFP and hexon signals per nucleus were
242 measured, and infected nuclei classified using the median GFP or hexon signals per nucleus.
243 Subsequently, mean and standard deviation (SD) over all infected nuclei per well were
244 calculated in R version 3.3.2 (126). Data were plotted in GraphPad (GraphPad Software, Inc,
245 version 8.1.2).

246

247 **Transmission electron microscopy**

248 A549 ATCC cells grown on Alcian Blue-treated cover slips were infected with HAdV-C2-dE3B-
249 GFP in supplemented medium with 0, 1.25 or 3 μ M Nelfinavir and cultured for 40 h at standard
250 cell culture conditions. The samples were washed with ice-cold 0.1 M cacodylate buffer (pH 7.4)
251 and fixed at 4°C in 0.1 M ice-cold cacodylate buffer (pH 7.4), supplemented with 2.5% (v / v)
252 glutaraldehyde and 0.5 mg / ml ruthenium red for 1 h. Cells were washed with 0.1 M cacodylate
253 buffer (pH 7.4) and post-fixed at RT in 0.05 M cacodylate buffer (pH 7.4) supplemented with
254 0.5% (v / v) OsO₄ and 0.25 mg / ml ruthenium red for 1 h. Following washing with 0.1 M
255 cacodylate buffer (pH 7.36) and H₂O, the samples were incubated in 2% (v / v) uranyl acetate at
256 4°C over night (ON). The samples were dehydrated in acetone and embedded in Epon as
257 described in (127). 85 nm slices were obtained (Leica Ultracut UCT, Leica, Wetzlar, Germany)
258 and stained with uranyl acetate.

259

260 **HAdV-C5 virus production in presence of Nelfinavir**

261 HAdV-C5 was amplified in the medium containing 0, 1.25 or 3 μ M Nelfinavir for 4 days. Cells
262 were harvested and disrupted by three freeze / thaw cycles. The cell debris was removed by
263 Freon extraction and mature full HAdV virions were purified by two rounds of CsCl gradient
264 ultracentrifugation (106). Protein concentration was determined by BCA assay (Pierce BCA
265 Protein Assay Kit, Thermo Fisher Scientific, Waltham, USA). For long-term storage, virus stocks
266 were supplemented with 10% (v / v) glycerol and kept at -80°C.

267

268 **Negative staining electron microscopy**

269 Double CsCl gradient-purified HAdV particles were adhered to Collodion and 2% (v / v) amyl
270 acetate film-covered grids (300 mesh Formvar/carbon-supported copper support films, Electron
271 Microscopy Sciences, Hatfield, USA). Viral particles were negatively stained with 2% (v / v)
272 uranyl acetate and viewed on a transmission electron microscope (Philips CM100, Philips,
273 Amsterdam, Netherlands) at 100 kV. Images were acquired using a CCD camera (Orius
274 SC1000 with 4,000 x 2,600 pixels, Gatan, Pleasanton, USA).

275

276 **Western blot analysis of HAdV protease activity**

277 Double CsCl-purified grown in presence / absence of Nelfinavir (HAdV-C5^{±Nelfinavir}) stocks and
278 size standard (PageRuler plus, Thermo Fisher Scientific, Waltham, USA) were size-separated
279 on 12% acrylamide gel under reducing conditions and transferred to a PVDF membrane. HAdV
280 proteins were detected using the following primary antibodies: 1:10,000 R72 rabbit α -fiber (128),

281 1:1,000 rabbit α -pVI/VI (51), 1:1,000 R3 rabbit α -pVII/VII (Ulf Pettersson of Uppsala University)
282 and visualized using a goat α -rabbit-HRP (7074, Cell Signaling Technology, Danvers, USA) and
283 ECL Prime Western Blotting Detection Reagent (GE Health Care, Pittsburgh, USA). The
284 membranes were luminescence imaged on an Amersham Imager 680 (GE Health Care,
285 Pittsburgh, USA).

286

287 **Determination of nuclear size**

288 Infection and Nelfinavir treatment of A549 cells were performed as described under Microscopic
289 plaque assay with a cell seeding density of 15,000 cells / well. Wells were imaged with IXM-C
290 automated high-throughput fluorescence microscope (Molecular Devices, San Jose, USA) using
291 a 40x objective (NA 0.95) at confocal mode (62 μ m pinhole). DAPI channel was acquired for
292 nuclear Hoechst staining, FITC / GFP channel was acquired for viral GFP, TRITC / Texas red
293 channel was acquired for immunofluorescence ADP staining and Cy5 channel was acquired for
294 NHS-ester signal. 30 z steps with 0.5 μ m step size were acquired for each channel and maximal
295 projections were calculated. Image analysis was performed using CellProfiler (125). Nuclei
296 areas were segmented based on thresholded Hoechst signal. Infected cells were classified
297 based on a fixed threshold for median nuclear GFP intensity. Data processing was performed in
298 R version 3.3.2 (126). Statistical analysis was performed in GraphPad (GraphPad Software, Inc,
299 version 8.1.2) using the non-parametric Kolmogorov-Smirnov test.

300

301 **Cell binding assay of virus**

302 A549 cells were seeded at 7,500 cells per 96-well in full DMEM and allowed to attach over night
303 at standard cell culture conditions. The next day, the medium was replaced by 3×10^8 VP/ well of
304 double CsCl-purified HAdV-C5 ^{\pm Nelfinavir} virus stocks in 100 μ l ice-cold supplemented medium and
305 kept on ice for 30 min. Following a 15 min entry phase under standard cell culture conditions the
306 cells were fixed and the nuclei stained for 1 h at RT by addition of 33 μ l 16% PFA and 4 μ g / ml
307 Hoechst 33342 (Sigma-Aldrich, St. Louis, USA) in PBS. Following the above described immuno-
308 fluorescence staining procedure, the cell-bound HAdV virions were stained using 9C12 mouse
309 α -hexon (developed by Laurence Fayadat and Wiebe Olijve, obtained from Developmental
310 Studies Hybridoma Bank developed under the auspices of the National Institute of Child Health
311 and Human Development and maintained by the University of Iowa, Iowa City, USA) (129) and
312 goat α -mouse AlexaFluor488 (A11029, Thermo Fisher Scientific, Waltham, USA). Total area
313 was identified by Alexa-Fluor647 NHS ester staining (A20006, Thermo Fisher Scientific,
314 Waltham, USA). Max projections of confocal z-stacks (25 z steps spaced 1 μ m) were acquired
315 on a SP5 resonant APD (Leica, Wetzlar, Germany) at 1.7x zoom using a 63x glycerol objective
316 (numerical aperture 1.4).

317

318 **Assessment of HAdV infectivity of HAdV-C5 ^{\pm Nelfinavir}**

319 Fifteen thousand A549 cells were seeded per 96-well in full DMEM and allowed to attach over
320 night at standard cell culture conditions. The next day, the medium was replaced by double
321 CsCl-purified HAdV-C5 ^{\pm Nelfinavir} virus stocks at 50 to 0.001 pg / well of BCA-based viral protein
322 concentration and incubated at standard cell culture conditions. Cells were fixed at 52 hpi,
323 stained for HAdV hexon expression and imaged following the procedure described under

Image-based plaque assay. Images were quantified using Plaque2.0 (101). Nuclei were segmented based on Hoechst signal. Infected cells were segmented based on hexon immunofluorescence staining signal.

Egress assay

A549 cells were seeded at 480,000 cells per 6-well in full DMEM and infected at 1,100 pfu HAdV-C2-dE3B-GFP per well the next day. Following 1 h of warm incubation, the supernatant was removed, and cells were washed with PBS and detached by trypsin digestion. Infected cells were centrifuged and resuspended in fresh medium to remove any unbound input virus and seeded at 180,000 cells / 12-well in medium supplemented with 1.25, 3 or 10 μ M Nelfinavir or equivalent amounts of DMSO solvent control. At the indicated times pi, the supernatant was harvested and cleared by centrifugation. 200 μ l PBS / well was added to the infected monolayer. Cells were disrupted by three freeze / thaw cycles and freon extraction was performed. Supernatant and cell lysate were stored at 4°C until titration on naive A549 cells. PFA-fixed, Hoechst-stained cells were imaged at 44 hpi using a 4x objective (NA 0.20) on an epifluorescence IXM-XL (Molecular Devices, San Jose, USA). GFP-positive infected cells were classified based on median nuclear GFP intensity using automated image analysis by CellProfiler (125).

Quantification of infectious progeny production

Four hundred and eighty thousand A549 cells were seeded per 6-well dish and inoculated with 1,100 pfu HAdV-C2-dE3B-GFP / well for 1 h at 37°C, washed with PBS and detached by trypsin digestion. Infected cells were centrifuged and resuspended in fresh medium to remove any unbound input virus. Cells were seeded at 180,000 cells / 12-well in medium supplemented with 1.25, 3 or 10 μ M Nelfinavir or the respective DMSO solvent control. Viral progeny in the cell monolayer and supernatant was harvested at the indicated time pi by three freeze / thaw cycles. The lysates were cleared by centrifugation and stored at 4°C until titration on naive A549 cells. PFA-fixed, Hoechst-stained cells were imaged at 44 hpi using a 4x objective on an epifluorescence IXM-XL (Molecular Devices, San Jose, USA). GFP-positive infected cells were classified based on median nuclear GFP intensity using automated image analysis by CellProfiler (125). The yield per 12-well was extrapolated by linear regression of the number of infected cells per μ l of harvested whole well lysate using GraphPad (GraphPad Software, Inc, version 8.1.2).

Quantification of the antiviral potency of Nelfinavir

Infection was performed as described under Microscopic plaque assay. Cells were incubated with an inoculum ranging between 10 - 2,560 pfu / well HAdV-C2-dE3B-GFP for 1 h at 37°C. Cells were washed with PBS and 100 μ l DMEM phenol-free medium (Thermo Fisher Scientific, Waltham, USA), supplemented with 1% penicillin streptomycin (Sigma-Aldrich, St. Louis, USA), 1% L-glutamine (Sigma-Aldrich, St. Louis, USA), 7.5% FBS (Invitrogen, Carlsbad USA), 1% non-essential amino acids (Sigma-Aldrich, St. Louis, USA), 1% 100 mM sodium pyruvate (Thermo Fisher Scientific, Waltham, USA), 0.25 ng / ml Hoechst 33342 (Sigma-Aldrich, St. Louis, USA) and 1 μ g / ml propidium iodide (PI, Molecular Probes, Eugene, USA). Plates were

367 imaged at the indicated times pi on an IXM-C automated high-throughput fluorescence
368 microscope (Molecular Devices, San Jose, USA) using a 40x objective (NA 0.95) at confocal
369 mode (62 μ m pinhole). DAPI channel was acquired for nuclear Hoechst staining, FITC / GFP
370 channel was acquired for viral GFP and Cy5 channel was acquired for PI signal. 30 z steps with
371 0.5 μ m step size were acquired for each channel and maximal projections were calculated.

372

373 **Morphological plaque characterization**

374 Plaques were segmented in Plaque2.0 (101) and plaque region eccentricity was measured as
375 fraction of the distance between the two focal points of the ellipse divided by the length of the
376 major axis. Only plaque regions consisting of at least five infected cells ($\geq 6,000$ px²) with a
377 centroid located 600 px from the well rim were considered to exclude spatial limitations. Plaque
378 roundness was calculated as $1 - \text{eccentricity}$ (Equation 1).

$$\text{roundness} = 1 - \frac{4\pi * \text{area}}{\text{perimeter}^2} \quad \text{Equation 1}$$

379 Statistical analysis was performed in GraphPad (GraphPad Software, Inc, version 8.1.2) using
380 the non-parametric Kolmogorov-Smirnov test.

381

382 **Confocal microscopy of ADP localization**

383 Infection and immunofluorescence stainings were performed as described under Microscopic
384 plaque assay with a cell seeding density of 3,000 cells / well. Cells were incubated with 1:1,000
385 rabbit α -HAdV-C2-ADP₈₇₋₁₀₁ antibody (107) and subsequently stained using donkey α -rabbit-
386 AlexaFluor594 (21207, Thermo Fisher Scientific, Waltham, USA) and 0.2 μ g/ml NHS ester (Life
387 Technologies, Carlsbad, USA) for whole cell outline. Plates were imaged on an IXM-C
388 automated high-throughput fluorescence microscope (Molecular Devices, San Jose, USA) using
389 a 40x objective (NA 0.95) at confocal mode (62 μ m pinhole). DAPI channel was acquired for
390 nuclear Hoechst staining, FITC / GFP channel was acquired for viral GFP, TRITC / Texas red
391 channel was acquired for immunofluorescence ADP staining and Cy5 channel was acquired for
392 NHS ester signal. 30 z steps with 0.5 μ m step size were acquired for each channel and maximal
393 projections were calculated. Image analysis was performed using CellProfiler (125). Nuclei and
394 whole cell areas were segmented based on thresholded Hoechst and NHS ester signal,
395 respectively. Nuclear rim was defined as 10 pixel-wide area around the nuclear border. Infected
396 cells were classified based on the whole cell 5% quantile GFP intensity. Whole cell and nuclear
397 rim mean TRITC / Texas red (detecting ADP) intensities as well as whole cell 5-pixel granularity
398 per infected cell were normalized by the according mean over all infected cells of the solvent
399 control. Data processing was performed in R version 3.3.2 (126). Statistical analysis was
400 performed in GraphPad (GraphPad Software, Inc, version 8.1.2) using the non-parametric
401 Kolmogorov-Smirnov test.

402

403 **Western blot analysis of ADP processing**

404 Four hundred and eighty thousand A549 cells were seeded per 6-well, incubated o/n and
405 inoculated with HAdV-C2-dE3B-GFP at 22,000 pfu / well in 1.2 ml full DMEM supplemented with
406 0 to 10 μ M Nelfinavir. Following 44 h of incubation in standard cell culture medium, cells were

407 placed on ice and the supernatant was removed. The cells were washed twice with ice-cold
408 PBS. Cells were lysed in 100 μ l COS lysis buffer (20 mM Tris-HCl pH 7.4, 100 mM NaCl, 1 mM
409 EDTA, 1% Triton X-100, 1 mM DTT, 25 mM β -Glycerophosphate disodium, 25 mM NaF, 1 mM
410 Na_3VO_4 , 1x protease inhibitors (Mini Complete, Roche, Basel, Switzerland) for 5 min on ice.
411 Supernatant and washing PBS were collected and cells pelleted by centrifugation at 16,000 xg
412 for 5 min at 4°C. Lysates were scraped off and used to resuspend the pelleted cells. Following
413 another centrifugation, the supernatant was collected and stored at -20°C. Samples of 15 μ l
414 lysate were supplemented with SDS-containing loading buffer (0.35 M Tris-HCl pH 6.8, 0.28%
415 SDS, 30 g / l DTT, 0.6 g / l bromophenol blue). Samples were denatured at 95°C for 5 min and
416 proteins were separated on a denaturing 15% acrylamide gel. Proteins transferred to a PVDF
417 membrane were detected with 1:1,000 of a rabbit α -HAdV-C2 ADP₇₈₋₉₃ antibody (107) followed
418 by goat α -rabbit-HRP (7074, Cell Signaling Technology, Danvers, USA). Protein bands were
419 visualized using ECL Prime Western Blotting Detection Reagent (GE Health Care, Pittsburgh,
420 USA) and luminescence imaged on an Amersham Imager 680 (GE Health Care, Pittsburgh,
421 USA).

422

423 **Neutralization of HAdV cell-free progeny**

424 A549 cells were seeded at 15,000 cells per well of a 96-well-plate, incubated o/n and inoculated
425 with HAdV-C2-dE3B-GFP at 34 pfu / well for 1 h at 37°C. Virus was removed and cells were
426 washed with PBS, before 0.25 ng / ml Hoechst (Sigma-Aldrich, St. Louis, USA)-supplemented
427 DMEM medium containing 1:12 HAdV-C2/5-neutralizing dog serum, kindly supplied by Anja
428 Ehrhardt, University Witten/Herdecke, Germany (130), supplemented with 40% v / v glycerol),
429 control goat serum (Thermo Fisher Scientific, Waltham, USA, supplemented with 40% v / v
430 glycerol) or the corresponding volume glycerol only. Cells were imaged using a 4x objective (NA
431 0.20) on an epifluorescence IXM-XL microscope (Molecular Devices, San Jose, USA).

432

433 **Crystal violet-stained plaques**

434 Plaque shapes were also assessed by conventional crystal violet-stained plaque assay,
435 performed in A549 cells in liquid supplemented DMEM medium. All infections were performed at
436 37°C, 95% humidity and 5% CO₂ atmosphere. At the indicated time pi, cells were fixed and
437 stained for 60 min with PBS solution containing 3 mg / ml crystal violet and 4% PFA added
438 directly to the medium from a 16% stock solution. Plates were de-stained in H₂O, dried and
439 imaged using a standard 20 mega pixel phone camera under white light illumination.

Results

Nelfinavir is a non-toxic, potent inhibitor of HAdV-C multicycle infection

An accompanying paper describes a full cycle, image-based screen of 1,278 out of 1,280 PCL compounds against HAdV-C2-dE3B-GFP, where Clopamide and Amphotericin B were excluded due to precipitation during acoustic dispensation into the screening plates (100). The screen was conducted in adenocarcinomic human alveolar basal epithelial (A549) cells at 1.25 μM compound concentration, and identified Nelfinavir, Aminacrine, Dequalinium dichloride and Thonzonium bromide as hits (Supplementary Table 1). Nelfinavir (CAS number 159989-65-8), hereafter referred to as Nelfinavir, strongly inhibited plaque numbers at nanomolar concentrations, comparable to the known HAdV nucleoside analogue inhibitor 3'-deoxy-3'-fluorothymidine (DFT, Figure 1A, 1B). Dequalinium dichloride, Aminacrine and Thonzonium bromide were excluded from further analyses due to toxicity (100), and potential mutagenic effects (131). Long-term incubations of uninfected A549 cells with Nelfinavir up to 115 h showed median toxicity TC_{50} of 25.7 μM , as determined by cell impedance measurements using xCELLigence (Figure 1C). xCELLigence measures the impedance of electrical currents imposed by cell adherence to gold-plated microelectrodes implanted in culture wells. Impedance is expressed as cell index (CI), a unitless parameter proportional to the cell number, cell size, and cell adherence. For raw CI profiles, see Supplementary Figure 1A. CI measurements were consistent with presto-blue assays, and cell numbers determined by counting nuclei (Supplementary Table 1). This was in agreement with previous reports, and acceptable side effects in clinical use against HIV (102, 132). The therapeutic index 50 (TI_{50}) of Nelfinavir was 27.1 (Figure 1D), as determined by the ratio between the concentration yielding 50% loss of cell nuclei ($\text{TC}_{50} = 10.01 \mu\text{M}$) and the effective concentration yielding 50% inhibition of fluorescent plaque formation ($\text{EC}_{50} = 0.37 \mu\text{M}$). The data indicates that Nelfinavir is an effective, non-toxic inhibitor of HAdV-C2 multi-cycle infection.

Nelfinavir does not affect single round infection

We first tested if Nelfinavir affected viral protein production. HAdV-C2-dE3B-GFP-infected A549 cells were analysed for GFP under the immediate early CMV promoter, and the late protein hexon expressed after viral DNA replication at 46 hours post infection (hpi). Results indicate that Nelfinavir had no effect on GFP or hexon expression at the tested concentrations, while the formation of fluorescent plaques was completely inhibited (Figure 2A, and Figure 1D). This result was in agreement with the notion that Nelfinavir did not affect the replication of the HAdV-C5 genome, as determined by titration of cell-associated infectious particles (133). We next examined if Nelfinavir affected the formation of viral particles. Transmission electron microscopy (TEM) of HAdV-C2-dE3B-GFP-infected cells revealed large numbers of virions in the nuclei of Nelfinavir-treated and untreated cells (Figure 2B). This result was conforming with the observation that the nuclei of Nelfinavir-treated cells expanded in area over time, indistinguishable from control cells (Supplementary Figure 1B).

To test if Nelfinavir affected virion maturation, we analysed purified virions by SDS-PAGE and Western blotting against proteins pVI/VI and pVII/VII using previously characterized antibodies.

483 There was no evidence for increase of precursor VI or VII (pVI or pVII) in HAdV-C5 from
484 Nelfinavir-treated cells, in contrast to temperature-sensitive (ts) 1 particles, which lack the
485 L3/p23 protease due to the point mutation P137L in p23 (134) (Figure 2C). This showed that
486 Nelfinavir did not affect the proteolytic maturation of the virus by the L3/p23 cysteine protease.
487 In accordance, purified HAdV-C5 from Nelfinavir-treated cells attached to naive A549 cells and
488 gave rise to viral gene expression as effectively as control HAdV-C5 particles (Figures 2D, 2E).
489 Together, these results indicate that Nelfinavir does not affect the production of infectious
490 virions in single round infections.

491

492 **Nelfinavir inhibits HAdV-C egress**

493 We investigated the kinetics of HAdV-C2-dE3B-GFP production and the release to the
494 supernatant. Supernatants and whole cell lysates of treated- and non-treated infected cells were
495 harvested at different time points (Figure 3A). At 44 hpi, cell lysates of Nelfinavir and control
496 cells contained similar infectivity, but did not yet release virus to the supernatant, as shown by
497 titration on naive A549 cells. At 72 or 120 hpi, control cells, but not Nelfinavir-treated cells had
498 released virus to the supernatant. Notably, the viral titer in the supernatant of control cells at 120
499 hpi was so high, that nearly all the cells in the indicator plates dissociated from the plates. The
500 difference in infectious load was confirmed by titration of supernatants from separate time
501 course experiments at three different concentrations of Nelfinavir (Figure 3B). At 7 dpi, a dosage
502 of 1.25 μ M reduced the total yield of infectious particles in the supernatant by three orders of
503 magnitude, underscoring the potency of Nelfinavir to block the dissemination of HAdV-C-dE3B-
504 GFP. Moreover, Nelfinavir limited HAdV-C2 transmission when added as late as 40 hpi (Figure
505 3C). These findings indicate that Nelfinavir impairs the egress of progeny from the host cell.

506

507 We next assessed the potency of the Nelfinavir against HAdV-C2 transmission by quantifying
508 the number of nuclei, which normally decreases due to lytic virus replication. Nelfinavir (3 μ M)
509 robustly reduced the number of dead cells, and strongly reduced the number of infected cells up
510 to 100 pfu / well (Figure 3D). Remarkably, HAdV-C2-dE3B-GFP formed delayed plaques in
511 presence of Nelfinavir, starting at 4 dpi (Figures 3E, 3F). These late plaques showed a strikingly
512 round morphology, which was calculated to be significantly different from the comet-shaped
513 plaques early in infection of control cells (Figure 3G). The direction of the comet tail of lytic
514 plaques can be aligned by tilting of the incubation plate (72). Thereby, the cell monolayer is
515 positioned non-orthogonally to the vector of thermal convection flux of the liquid cell culture
516 medium. While the direction of the comet-shaped plaques could be aligned using this method in
517 the non-treated infections, the late Nelfinavir plaques remained mostly round (Supplementary
518 Figures 2A-C). Moreover, there was no correlation between the size of the plaques and their
519 roundness irrespective of Nelfinavir up to 7 dpi, demonstrating that the round plaques did not
520 change morphology over time (Supplementary Figure 2D). Collectively, the data indicate that
521 virus transmission in presence of Nelfinavir is not driven by the bulk current of cell free medium.

522

523 **HAdV inhibition by Nelfinavir depends on ADP**

524 ADP is expressed at high levels late in infection and enhances cell lysis (94, 135). To test if
525 ADP was required for Nelfinavir inhibition of lytic spread, we generated an ADP-depleted HAdV-

526 C2-dE3B-GFP mutant, HAdV-C2-dE3B-GFP-dADP. The mutant completely lacks ADP
527 expression, as indicated by immunofluorescence and Western blot experiments (Supplementary
528 Figure 3A, 3B). HAdV-C2-dE3B-GFP-dADP formed particles indistinguishable from HAdV-C2-
529 dE3B-GFP, as indicated by negative stain EM (Supplementary Figure 3C). HAdV-C2-dE3B-
530 GFP-dADP showed a delayed onset of plaque formation by about 1 day, compared to HAdV-
531 C2-dE3B-GFP (Figure 4A). These data are in agreement with previous kinetic studies with the
532 ADP deletion mutant HAdV-C dl712 (107) (see also Supplementary Figure 3A). HAdV-C2-
533 dE3B-GFP-dADP plaques were comet-shaped, albeit their comet-heads appeared bigger and
534 more dense (Figure 4A). While the parental virus was highly sensitive to Nelfinavir, HAdV-C2-
535 dE3B-GFP-dADP required much higher concentrations of the compound to show inhibition of
536 plaque formation (Figure 4B, Supplementary Table 2). In accordance, the ADP-deleted virus
537 induced cell death independent of Nelfinavir, unlike the ADP-expressing virus, as concluded
538 from cell impedance measurements with xCELLigence (Figure 4C, Supplementary Figures 3D,
539 3E). Finally, HAdV-C2-dE3B-GFP-dADP exhibited a strongly diminished separation of anti-viral
540 efficacy from toxicity, as indicated by reduced TI_{50} values compared to the parental virus, for
541 example 2.1 versus 66.8 with A549 cells, 8.9 versus 61.0 with HeLa cells, and 4.6 versus 55.2
542 with HBEC cells (Figure 4D). These effects were in agreement with similar experiments
543 performed with the previously described ADP-knock out mutant dl712 and the parental rec700,
544 an HAdV-C5/2 hybrid virus (135, 136). The data are shown in (Supplementary Figures 3F to
545 3H). Together, the results show that the selective antiviral effects of Nelfinavir are more cell-type
546 dependent in case of HAdV lacking ADP than in ADP-expressing viruses, and the effects are
547 comparatively small for viruses lacking ADP.

548

549 Finally, we performed immunofluorescence experiments with HAdV-C2-dE3B-GFP-infected
550 A549 cells at 44 hpi (Figure 4E). Under non-perturbed conditions, ADP accumulated in cytoplas-
551 mic foci and the nuclear envelope. Nelfinavir treatment did not affect the overall ADP expression
552 levels nor the amount of ADP in the nuclear periphery, including the nuclear envelope, but
553 completely abolished the cytoplasmic ADP foci as indicated by granularity quantifications
554 (Figure 4E, right graph). Intriguingly, Tollefson and co-workers observed earlier that ADP
555 lacking luminal O-glycosylation sites did not localize to large cytoplasmic granules and the
556 corresponding HAdV-C mutant pm734.4 was non-lytic (107). We speculate that the localization
557 of ADP in cytoplasmic organelles, such as Golgi compartments, where O-glycosylation occurs
558 (137), could enhance the cell lytic function of ADP. Together, the data show that ADP is a major
559 susceptibility factor for inhibition of HAdV-C infection spread by Nelfinavir.

560

561 **A round non-lytic plaque phenotype in HAdV-C infection**

562 Viruses are transmitted between cells by three major mechanisms, cell-free through the extra-
563 cellular medium, directly from cell-to-cell, or in an organism by means of infected motile cells or
564 fluid flow in blood or lymphoid vessels. This can result in far-reaching or mostly local virus dis-
565 semination (for a simplified cartoon, see Figure 5A). In cell culture, HAdV-C transmission from a
566 lytic infected cell (staining PI-positive) yields comet-shaped infection foci due to convective
567 passive mass flow in the cell culture medium (72, 101), consistent with lytic HAdV-C infection
568 (74, 135). In accordance, neutralizing antibodies against HAdV-C2 added to the cell culture

569 medium suppressed the comet-shaped plaques of HAdV-C2-dE3B-GFP, and yielded confined,
570 predominantly round-shaped infection foci 4 dpi, akin to Nelfinavir-treated infections (Figure 5B).

571

572 To test if round-shaped infection foci (plaques) occurred in regular HAdV-C2-dE3B-GFP
573 infections, we analysed A549 cells infected with less than one plaque forming unit (pfu) per well
574 in 160 wells up to 8 dpi. Thirty three wells developed a single plaque. Twenty four of them were
575 fast emerging comet-shaped plaques, of which the donor cell (indicated by the pink arrow),
576 disappeared between 2 and 3 dpi (Figure 5C, upper panel). In contrast, nine wells developed
577 delayed round plaques starting 6 dpi (Figure 5D, lower panel). In all these cases, the original
578 infected cell (orange arrow) remained GFP-positive and apparently viable, and the surrounding
579 cells gradually became infected. These data suggest that HAdV-C2 utilizes both lytic and non-
580 lytic transmission, the former involving cell-free transmission, and the latter cell-associated
581 transmission.

582

583 **Nelfinavir has a broad anti-HAdV spectrum**

584 We finally assessed the inhibition breadth of Nelfinavir against various HAdV types from species
585 A, B, C and D in different human cell lines, as well as mouse adenovirus (MAV) 1 and 3 in
586 mouse rectum carcinoma CMT93 cells. To balance statistical significance and automated
587 plaque segmentation, we first determined the optimal amount of inoculum and duration of
588 infection for each virus and cell line. The resulting TI_{50} values of Nelfinavir were heterogeneous
589 for different HAdV types, as determined in A549 cells (Figure 6A, for details see Supplementary
590 Table 2). While all the tested HAdV-C types as well as HAdV-B14 showed high TI_{50} s (>10)
591 ranging from 12.22 (HAdV-C1) to 71.09 (HAdV-C2). Members of HAdV species A, D and most
592 of the HAdV-B types showed intermediate (2 - 10) to low Nelfinavir susceptibility (<2), notably
593 HAdV-B7 and B11 with TI_{50} <1. MAV-1 and 3 also showed low susceptibility. Noticeably, a high
594 susceptibility of HAdV-C was consistently observed in human lung epithelial carcinoma (A549)
595 cells, human epithelial cervix carcinoma (HeLa) cells, immortalized primary normal human
596 corneal epithelial (HCE) cells as well as normal human bronchial epithelial (HBEC) cells. The
597 corresponding TI_{50} values were in the same range as for herpes simplex virus (HSV) 1, for
598 which Nelfinavir was reported to be an egress inhibitor (133, 138, 139).

599

600 We finally examined the plaque morphologies in non-perturbed infections by immunofluores-
601 cence staining of the late proteins VI and hexon, as well as macroscopic analyses of crystal
602 violet stained dishes for classical plaques (Figure 6B). Viruses that were highly susceptible to
603 Nelfinavir (exhibiting high TI_{50} values) formed exclusively comet-shaped plaques. Viruses with
604 low TI_{50} values, such as A31, B11 or D37 had a high fraction of round plaques, even when
605 infected with more than 1 pfu / well. This demonstrates that the slowly growing round infection
606 foci observed in fluorescent microscopy gave similarly shaped lesions due to cytotoxicity, akin to
607 the lytic comet-shaped foci. We conclude that HAdV types employ lytic cell-free and non-lytic
608 cell-to-cell transmission modes and give rise to different plaque phenotypes.

609

610

611

612 Discussion

613

614 A phenotypic screen of the PCL identified Nelfinavir as a potent, post-exposure inhibitor of
615 HAdV-C2-dE3B-GFP plaque formation in cell culture (100). Nelfinavir is a non-nucleoside class
616 inhibitor against a range of HAdV types. Surprisingly, we found Nelfinavir to inhibit HAdV
617 infection, although Nelfinavir was previously classified as inactive against HAdV-C based on
618 replication assays (133). It is the off-patent FDA-approved active pharmaceutical ingredient of
619 Viracept. Nelfinavir was originally developed as an inhibitor against the HIV aspartyl protease. It
620 is orally bioavailable, with an inhibitory concentration in the low nanomolar range (102, 132).
621 Nelfinavir inhibits the replication of enveloped viruses, including SARS coronavirus (140),
622 hepatitis C virus (141) as well as α -, β - and γ -herpes viruses (133). In the case of the α -herpes
623 virus HSV-1, Nelfinavir inhibits the envelopment of the capsid with cytoplasmic membranes.
624 This coincided with impaired glycosylation of gB and gC in the TGN (133, 138, 139, 142).
625 Nelfinavir was reported to inhibit the activity of regulatory proteases in the Golgi, the growth of
626 cancer cells and to induce a wealth of other effects, including autophagy, ER stress, the
627 unfolded protein response, and apoptosis (143–149) (150–152, reviewed in 153–155). It
628 remains unknown if Nelfinavir exerts these pleiotropic effects by interfering with diverse
629 processes or a particular one.

630

631 Here, we demonstrate that Nelfinavir inhibits the egress of HAdV particles without perturbing
632 other viral replication steps including entry, assembly and maturation. Morphometric analyses of
633 the fluorescent plaques indicated that HAdV-C propagates by two distinct mechanisms, lytic and
634 non-lytic. Lytic transmission led to comet-shaped convection driven plaques, whereas non-lytic
635 transmission gave rise to symmetric round-shaped plaques. Nelfinavir specifically suppressed
636 the lytic spread of HAdV, most prominently the HAdV-C types and B14, but not other HAdV,
637 such as A31 or D37. Incidentally, HAdV-C and B14 replicate to considerable levels in Syrian
638 hamsters, whereas other HAdV types do not (31, 156, 157). We infer that lytic infection could be
639 a pathogenicity driver, at least in the hamster model.

640

641 The molecular mechanisms underlying cell lysis in AdV infection are not well understood, largely
642 due to the lack of specific assays and inhibitors. Single cell analyses combined with machine
643 learning start to identify specific features of lytic cells, such as increased intra-nuclear pressure
644 compared to non-lytic cells (158). The lysis induced by HAdV was suggested to involve
645 caspase-dependent functions, and necrosis-like features (99, 159, 160). The best characterized
646 factor in HAdV cell lysis is ADP, a small membrane protein encoded in HAdV-C (90, 91, 161).
647 ADP-deletion mutants show delayed onset of plaque formation (73, 135). Lysis is enhanced by
648 increased ADP levels and tuned by post-translational ADP processing (73, 74, 135). ADP has a
649 single signal/anchor sequence, and its luminal domain is N- and O-glycosylated. The N-
650 terminal segment is cleaved off in the Golgi lumen, and the membrane-anchored ADP localizes
651 to the inner nuclear membrane (92, 107, 135). Interestingly, two cysteine residues in the
652 cytoplasmic domain adjacent to the transmembrane segment are palmitoylated (107) (162). S-
653 palmitoylation is known to support anchorage and sorting of host and viral membrane proteins.

654 Accordingly, S-palmitoylation in the Golgi facilitates protein oligomerization, virion assembly and
655 entry, as shown for structural proteins of enveloped viruses, including SARS-CoV-1 S, vesicular
656 stomatitis virus G, sindbis virus E2, influenza virus HA, respiratory syncytial virus F, or rubella
657 virus E1 and E2, as well as viroporin-mediated membrane permeabilization, including mouse
658 hepatitis virus E protein, SARS-CoV-1 E protein and sindbis virus 6K. For reviews, see (163,
659 164).

660

661 Conspicuously, the cell lysis defective HAdV mutant pm734.4 encodes a C2 mutant ADP with
662 two point mutations in the transmembrane domain, C₅₃R and M₅₆L (107). The mutant ADP
663 localizes to the ER and the nuclear envelope, but not the Golgi, unlike the parental wild type
664 virus rec700. The localization of the pm734.4 ADP is akin to the localization of HAdV-C2 ADP in
665 Nelfinavir-treated cells, which resist lysis and lack ADP localization in the Golgi. We speculate
666 that the palmitoylation of ADP in the Golgi is crucial for ADP to enhance the rupture of the
667 nuclear membrane in lytic HAdV-C egress. Nelfinavir may interfere with ADP palmitoylation
668 either by inhibiting a palmitoyl-acyltransferase or by dispersing the donor substrate for protein
669 palmitoylation, palmitoyl-coenzyme A (164). Remarkably, Nelfinavir has a high logP value, 4.1
670 to 4.68 (165, 166), and partitions into lipophilic domains of the cell, including membranes. This
671 is akin to another lipophilic drug with pleiotropic effects, the anti-viral and anti-helminthic
672 compound Niclosamide, which is a weak acid and acts as a protonophore extracting protons
673 from acidic organelles, and thereby inhibits virus entry and uncouples mitochondrial proton
674 gradients (167, 168).

675

676 We noticed that ADP is not the sole lysis factor of HAdV. HAdV types lacking ADP, such as B
677 types, also release their progeny by lysis, albeit with efficacies that vary depending on the cell
678 type (169–171). This is in agreement with the observation that HAdV types of the A, B and D
679 species form comet-shaped plaques, and that ADP-deleted HAdV-C2 lyse the host cell, and
680 form comet-shaped plaques, albeit delayed and with lower efficacy than ADP-containing rec700
681 or HAdV-C2-dE3B-GFP. Conspicuously, other AdV proteins besides ADP were reported to
682 interfere with cell lysis, such as the early region 4 ORF4 protein, which induces nuclear
683 envelope blebbing and promotes the loss of nuclear integrity (172, 173). This, together with
684 diverse cellular mechanisms underlying force generation and membrane rupture, could
685 compensate for the lack of ADP in some forms of lytic virus egress (51, 55, 173). We consider it
686 unlikely that genetic variability of the inoculum accounts for the presence of lytic and non-lytic
687 pathways, since the inoculum was derived from an infectious DNA clone of HAdV-C2-dE3B-
688 GFP, and lacked any mutations affecting amino acid coding across many passages (72).

689

690 In addition to providing a new inhibitor of lytic HAdV propagation, Nelfinavir revealed an alterna-
691 tive non-lytic HAdV transmission pathway, which gives rise to slow-growing symmetrical
692 plaques. This non-lytic pathway exists in unperturbed cells, but is camouflaged by the rapid and
693 far-reaching lytic infection. The non-lytic egress pathway is likely a deterministic process. It is
694 stable for at least eight days (see Fig. 5C). It remains to be explored if cells can switch between
695 the lytic and the non-lytic pathway. Regardless, non-lytic egress from the nucleus bypasses the
696 nuclear envelope and the plasma membrane. We speculate that the non-lytic pathway involves

697 sorting of HAdV particles to membrane sites where outward budding and scission occur. HAdV
698 budding through the nuclear envelope could involve the WASH complex, akin to nuclear release
699 of large RNPs in *Drosophila*, and perhaps similar to HSV budding (174, 175). Cytoplasmic
700 membrane budding could be enhanced by the ESCRT complex, which is known to release
701 enveloped viruses, such as HIV, and also facultative-enveloped viruses, such as hepatitis A
702 virus (176–178). Alternatively, autophagy could sequester virions from the nucleus and upon
703 fusion with the plasma membrane release virions from infected cell.

704

705 In conclusion, our work opens new therapeutic options for treating adenovirus disease, including
706 acute and persistent infections. For example, HAdV-C persists in lymphocytes, which resist lytic
707 infection, but also in epithelial cell lines under the repression of interferon and activation of the
708 unfolded protein response sensor IRE-1a (122, 123, 179–182). Nelfinavir might be considered
709 for anti-HAdV therapy, for example prophylactically in hematopoietic stem cell recipients, whose
710 life is threatened by reactivation of HAdV-C (5, 6, 183).

711 **Contributions**

712 UFG, VA and AY conceived the project. FG, VA, RW, LY, MG and NM performed experiments.
713 FG, VA, RW, LM, FK, VP, AY, GT, UFG analysed data. SH contributed essential viruses. FG
714 and UFG wrote the manuscript.

715
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725
726 **Conflict of interest**

727 The authors declare no conflict of interest.

728
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733
734 **Abbreviations**

735 ADP, adenovirus death protein;
736 AdV, adenovirus;
737 CAR, coxsackievirus adenovirus receptor;
738 CMV, cytomegalovirus;
739 DFT, 3'-Deoxy-3'-fluorothymidine;
740 DMEM, Dulbecco's Modified Eagle medium;
741 dpi, day(s) post infection;
742 EC₅₀, 50% effective concentration;
743 GFP, green fluorescent protein;
744 HAdV, human adenovirus;
745 HIV, human immunodeficiency virus;
746 hpi, hour(s) post infection;
747 HSV, herpes simplex virus;
748 kbp, kilo base pairs,
749 MAdV, mouse adenovirus;
750 o/n, over night;
751 ORF, open reading frame;
752 Nelfinavir Mesylate, Nelfinavir;
753 pBI, pBluescript;
754 PCL, Prestwick Chemical Library;

755 PFA, para-formaldehyde;
756 pfu, plaque forming unit(s);
757 pi, post infection;
758 PI, propidium iodide;
759 RT, room temperature;
760 SE, standard error;
761 SD, standard deviation;
762 TC₅₀, 50% toxic concentration;
763 TI, therapeutic index;
764 ts, temperature-sensitive;
765 VP, viral particles;
766 wt, wild type

767 **Figures**

768

769 **Figure 1: The small molecule Nelfinavir is a potent inhibitor of HAdV-C infection.**

770

771 **A** Representative 384-well epifluorescence microscopy images of cells treated with DMSO (left),
772 Nelfinavir (centre) and DFT (right), infected with HAdV-C2-dE3B-GFP for 72 h. Hoechst-stained nuclei are
773 shown in blue, viral GFP in green. Dotted lines indicate well outline. Scale bar is 5 mm.

774 **B** Structural formula of Nelfinavir mesylate.

775 **C** The half-maximal toxicity (TC_{50}) in uninfected A549 cells was determined by Nelfinavir dose-response
776 impedance measurements at different times of drug treatment. The x-axis indicates the time post cell
777 seeding, as well as drug addition. Impedance was recorded at intervals of 15 min using xCELLigence
778 reporting on the cell number and cell adhesion to the electrode-coated wells. The raw CI data are
779 available in Supplementary Figure 1.

780 **D** Separation of effect (EC_{50} , plaque numbers) and toxicity (TC_{50} , nuclei numbers) of Nelfinavir in A549
781 cells at 82 hpi based on four technical replicates. Plaque numbers per well are depicted as red circles,
782 and numbers of infected nuclei as green circles. Numbers of nuclei in Nelfinavir-treated, uninfected wells
783 are shown in blue; treated, infected wells shown in orange.

784 **Figure 2. Nelfinavir does not affect early or late steps of HAdV-C infection.**

785

786 **A** No effect of Nelfinavir on the expression of CMV-GFP (green) or the late viral protein hexon (red) in
787 HAdV-C2-dE3B-GFP-infected A549 cells. Data points represent for each of the four biological replicates:
788 mean median, nuclear intensities per well normalized to the mean median nuclear intensities of the
789 DMSO-treated wells. Epifluorescence microscopy images were segmented and analysed using
790 CellProfiler.

791 **B** Representative TEM images of late stage HAdV-C2-dE3B-GFP-infected A549 cells at 41 hpi reveal
792 viral particles inside the nucleus in both DMSO-treated and Nelfinavir-treated cells (white arrow head).
793 Black arrow heads indicate the nuclear envelope, arrow head with * points to rupture. Scale bar
794 equivalent to 2 μ m.

795 **C** Nelfinavir does not affect the maturation of HAdV-C5, as indicated by fully processed VI and VII
796 proteins in purified particles grown in presence of Nelfinavir. Note that HAdV-C2-ts1 lacking the L3/p23
797 protease contains the precursor capsid proteins of VI and VII (pVI and pVII).

798 **D** HAdV-C5 grown in presence of Nelfinavir (HAdV-C5^{+Nelfinavir}) binds to naive A549 cells similar as HAdV-
799 C5 from control cells. Cells were incubated with virus at 4°C for 1 h and fixed with PFA. Staining of viral
800 capsids with an α -hexon antibody (green puncta). Nuclei shown with Hoechst staining (blue). Cells were
801 visualized by NHS-ester staining (red). Images are max projections of confocal z stacks, and also show
802 zoomed in views (grey squares). Scale bars = 20 μ m.

803 **E** Particles produced in presence of Nelfinavir are fully infectious. A549 cells were inoculated with purified
804 HAdV-C5 and incubated in absence (grey) or presence of Nelfinavir (orange colors) for 44 hpi. Infection
805 analyses by α -hexon immunofluorescence staining, and cell numbers derived from Hoechst staining
806 (blue). Bars represent means of four technical replicates. Error bars indicate standard deviation.

807

808 **Figure 3. Nelfinavir is a post-exposure inhibitor of HAdV-C egress.**

809

810 **A** A549 cells were imaged at 3 days post inoculation with 1:10 diluted cell lysates (left) or supernatants
811 (right) from Nelfinavir or control A549 cells, which had been infected with HAdV-C2-dE3B-GFP for the
812 indicated times (harvested hpi). Results show delayed viral progeny release to the supernatant of
813 Nelfinavir-treated cells. Nuclei are shown in blue, infection marker in green (GFP).

814 **B** Released and cell-associated progeny from HAdV-C2-dE3B-GFP-infected A549 cells treated with
815 Nelfinavir (orange) or DMSO (green), as determined by titration on A549 cells in a 12-well assay format.
816 Lines indicate mean slopes, dotted lines standard error. Linear regression of three biological triplicates.

817 **C** Time-resolved emergence of plaques in HAdV-C2-dE3B-GFP-infected A549 cells treated with 1.25 μ M
818 Nelfinavir. Plaques in infected, non-treated wells are shown in green, Nelfinavir-treated wells in orange
819 and nuclei in blue. Data points represent one of eight technical replicates. Coloured vertical lines indicate
820 means and error bars the standard deviations.

821 **D** The inhibitory effect of Nelfinavir on HAdV-C2-dE3B-GFP spread is dependent on the amount input
822 virus during initial infection. Number of infected, GFP-positive cells shown in green at 3 μ M Nelfinavir
823 relative to the mean infection of solvent-treated cells infected with the corresponding dosage. Total
824 number of nuclei shown in blue, number of PI-positive dead cells in red. Note that the number of infected
825 cells at 43 hpi is not affected by the Nelfinavir treatment. Data points represent means of four technical
826 replicates. Dotted lines indicate standard deviation.

827 **E** Treatment of HAdV-C2-dE3B-GFP-infected A549 cells with 1.25 μ M Nelfinavir suppresses comet-
828 shaped plaques and reveals slow growing quasi-round plaques. Viral GFP expression levels are shown
829 as 16-color LUT. Scale bar is 1 mm.

830 **F** Treatment with 1.25 μ M Nelfinavir inhibits HAdV-C2-dE3B-GFP infection of A549 cells by slowing
831 plaque formation. The numbers of infected cells and plaques per well of DMSO-treated wells are shown
832 in green, those of Nelfinavir-treated wells in orange. Data points represent means of 24 technical
833 replicates, including the well shown in the micrographs of panel **D**. Error bars indicate standard deviation.
834 Statistical significance of drug versus non-treated cells was derived by the Kolmogorov-Smirnov test, p
835 value < 0.0001 (****).

836 **G** The delayed HAdV-C2-dE3B-GFP plaques in presence of 1.25 μ M Nelfinavir are significantly rounder
837 than control plaques, as indicated by Kolmogorov-Smirnov test. Data points indicate plaque regions in the
838 well centre harbouring a single peak region. Summary of 24 technical replicates including the well shown
839 in the micrographs of panel **D**. Regions consisting of at least 5 infected cells ($\geq 1,500 \mu\text{m}^2$) were
840 considered as a plaque. Plaque morphologies in control wells could not be quantified later than 3 dpi due
841 to rapid virus dissemination. Plaques from DMSO-treated cells 3 dpi compared to Nelfinavir-treated ones
842 5 dpi: approximate p value < 0.0001 (****). DMSO-treated plaques 3 dpi vs. Nelfinavir-treated plaques 6
843 dpi: approximate p value < 0.0001 (****). Statistical significance by Kolmogorov-Smirnov test.

844 **Figure 4. ADP contributes to the inhibitory effect of Nelfinavir against HAdV-C.**

845

846 **A** The deletion of ADP from HAdV-C2-dE3B-GFP delays plaque formation in A549 cells by one day, but
847 does not change plaque shape. Cells were infected with 1.1×10^5 VP / well. GFP is in green, hexon
848 staining red, Hoechst signal of nuclei blue. Scale bar is 1 mm.

849 **B** The deletion of ADP from HAdV-C2-dE3B-GFP reduces the antiviral effects of Nelfinavir in A549, with
850 $EC_{50} = 5.82$ compared to $0.22 \mu M$ for the parental virus. HAdV-C2-dE3B-GFP infection was quantified at
851 72 hpi, and 96 hpi for the ADP deletion mutant. Plaque numbers per well were normalized to the mean
852 DMSO control and depicted as full green triangles for HAdV-C2-dE3B-GFP and empty red triangles for
853 the ADP deletion mutant. Nuclei numbers of non-infected, treated wells were normalized to the mean
854 DMSO control and depicted as full blue circles (72 h incubation), and empty blue circles (96 h). Data
855 points represent means of four technical replicates. Error bars indicate standard deviation. EC_{50} values
856 were derived from non-linear curve fitting. For detailed information and statistics, see Supplementary
857 Table 2.

858 **C** The delay of cell death was calculated from the highest mean cell index (CI) and its half maximum for
859 each treatment (mean of two technical replicates). HAdV-C2-dE3B-GFP data in green and dADP in red.
860 For HAdV-C2-dE3B-GFP-infected A549 treated with $25 \mu M$ Nelfinavir, the measurement was aborted due
861 to overgrowth causing cytotoxicity before the maximal cell index was reached. Treatment with $100 \mu M$
862 Nelfinavir was toxic.

863 **D** Therapeutic index (TI_{50}) derived from the ratio of Nelfinavir concentration causing 50% toxicity (TC_{50})
864 and the concentration leading to 50% reduction in plaque numbers per well (EC_{50}). Results from different
865 cancer and primary cells are shown for HAdV-C2-dE3B-GFP and HAdV-C2-dE3B-dADP lacking ADP. For
866 detailed information and statistics, see Supplementary Table 2.

867 **E** Representative high-magnification confocal images of HAdV-C2-dE3B-GFP-infected A549 cells 44 hpi
868 showing the effect of Nelfinavir on ADP localization (left panel). ADP was stained by immunofluorescence
869 with a rabbit α -HAdV-C2-ADP₈₇₋₁₀₁ antibody (red). Cells were stained using NHS-ester (grey scale). White
870 arrow heads highlight infected cells. Nuclei (blue), viral GFP (green). Images are max projections of 30 z
871 planes with $0.5 \mu m$ z steps. Scale bar $10 \mu m$. Relative units (RU) of total ADP expression (grey),
872 localization to the nuclear rim (blue) and granularity (red) normalized to the mean values from DMSO
873 control cells (right panel). The data set is comprised of 20 Nelfinavir-treated infected cells, and 23 control
874 cells. Solid lines indicate median, dotted lines the 5-95% quantile. Kolmogorov-Smirnov indicated an ADP
875 granularity p value of 0.0019 (**).

876 **Figure 5: Round plaque phenotypes in presence of neutralising anti-HAdV-C2 antibodies and in**
877 **unperturbed HAdV-C2 infections.**

878

879 **A** Schematic overview of pathogen transmission routes in cell cultures. Cell lysis kills the donor cell and
880 releases progeny, while non-lytic egress preserves the infected donor cell. Convection in the media leads
881 to long-distance, comet-shaped plaques, and cell-free virus transmission is susceptible to neutralizing
882 antibodies. In contrast, direct cell-to-cell spread of virus gives rise to symmetric slow growing plaques,
883 resistant to neutralizing antibodies. Non-infected cells are shown in grey and nuclei in blue. First round
884 infected cells are shown in dark green, nuclei with a ruptured envelope in red. Second round infected
885 cells are shown in light green. Grey arrow represents direction of convective flow. Axes indicate side or
886 top-down views.

887 **B** Inhibition of cell-free HAdV-C2-dE3B-GFP transmission by an anti-HAdV-C2/5-neutralizing serum.
888 Nuclei are shown in blue, viral GFP in green.

889 **C** Infection of A549 cells with limiting amounts of HAdV-C2-dE3B-GFP (<1 pfu/ well, 9-75 VP/ well) in 160
890 wells gives rise to 33 single plaques / well. Twenty-four wells contained GFP-positive comet-shaped
891 plaques (upper panel), and nine developed delayed round plaques (lower panel). Dashed coloured
892 squares indicate magnified regions of first-round infected cell below. Infected cell leading to comet-
893 shaped plaque (upper panel, pink arrow) lyses at 3 dpi as indicated by loss of GFP signal. Infected cell
894 giving rise to round plaque (lower panel, orange arrow) remains GFP-positive. Scale bar is 1 mm.

895 **Figure 6: Susceptibility of HAdV to Nelfinavir correlates with plaque shape.**

896

897 **A** Therapeutic index (TI_{50}) calculated from the ratio of Nelfinavir concentration causing 50% toxicity (TC_{50})
898 and the concentration leading to 50% plaque reduction (EC_{50}). Different HAdVs, mouse adenoviruses
899 (MAdV) and herpes simplex virus 1 (HSV-1) were tested in different cancer and primary cell lines. For
900 detailed information and statistics, see Supplementary Table 2.

901 **B** Representative microscopic and macroscopic plaque morphologies of Nelfinavir-sensitive and
902 insensitive HAdV types. Grey scale images show plaques based on epifluorescence microscopy of hexon
903 immunostaining or GFP expression in A549 cells (96 well format). Scale bar is 1 mm. Coloured images
904 show plaques visualized by crystal violet staining in A549 cells (12 well format). Scale bar is 5 mm.

905 **References**

- 906
- 907 1. **Rowe WP, Huebner RJ, Gilmore LK, Parrott RH, Ward TG.** 1953. Isolation of a cytopathogenic
908 agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc*
909 *Exp Biol Med* **84**:570–573.
- 910 2. **Ismail AM, Zhou X, Dyer DW, Seto D, Rajaiya J, Chodosh J.** 2019. Genomic foundations of
911 evolution and ocular pathogenesis in human adenovirus species D. *FEBS Lett.* **593**:3583–3608.
- 912 3. **Harrach B, Tarján ZL, Benkő M.** 2019. Adenoviruses across the animal kingdom: a walk in the
913 zoo. *FEBS Lett.*
- 914 4. **Mei Y-F, Lindman K, Wadell G.** 2002. Human adenoviruses of subgenera B, C, and E with
915 various tropisms differ in both binding to and replication in the epithelial A549 and 293 cells.
916 *Virology* **295**:30–43.
- 917 5. **Lynch JP, Kajon AE.** 2016. Adenovirus: epidemiology, global spread of novel serotypes, and
918 advances in treatment and prevention. *Semin Respir Crit Care Med* **37**:586–602.
- 919 6. **Lion T.** 2019. Adenovirus persistence, reactivation, and clinical management. *FEBS Lett.*
- 920 7. **Krilov LR.** 2005. Adenovirus infections in the immunocompromised host. *Pediatr. Infect. Dis. J.*
921 **24**:555–556.
- 922 8. **Greber UF, Arnberg N, Wadell G, Benkő M, Kremer EJ.** 2013. Adenoviruses - from pathogens
923 to therapeutics: a report on the 10th International Adenovirus Meeting. *Cell Microbiol.* **15**:16–23.
- 924 9. **Bailey ES, Fieldhouse JK, Choi JY, Gray GC.** 2018. A mini review of the zoonotic threat
925 potential of influenza viruses, coronaviruses, adenoviruses, and enteroviruses. *Front Public*
926 *Health* **6**:104.
- 927 10. **Hwang S-M, Park D-E, Yang Y-I, Park S-J, Lee H-K, Kim M-J, Chun B-C.** 2013. Outbreak of
928 febrile respiratory illness caused by adenovirus at a South Korean military training facility: clinical
929 and radiological characteristics of adenovirus pneumonia. *Jpn J Infect Dis* **66**:359–365.
- 930 11. **Potter RN, Cantrell JA, Mallak CT, Gaydos JC.** 2012. Adenovirus-associated deaths in US
931 military during postvaccination period, 1999–2010. *Emerging Infect. Dis.* **18**:507–509.
- 932 12. **Erdman DD, Xu W, Gerber SI, Gray GC, Schnurr D, Kajon AE, Anderson LJ.** 2002. Molecular
933 epidemiology of adenovirus type 7 in the United States, 1966–2000. *Emerging Infect. Dis.* **8**:269–
934 277.
- 935 13. **State of New Jersey Department of Health.** 2019. Adenovirus. Communicable Disease Service
936 - Adenovirus.
- 937 14. **Deal C, Pekosz A, Ketner G.** 2013. Prospects for oral replicating adenovirus-vectored vaccines.
938 *Vaccine* **31**:3236–3243.
- 939 15. **Radin JM, Hawksworth AW, Blair PJ, Faix DJ, Raman R, Russell KL, Gray GC.** 2014.
940 Dramatic decline of respiratory illness among US military recruits after the renewed use of
941 adenovirus vaccines. *Clin. Infect. Dis.* **59**:962–968.
- 942 16. **Centers for Disease Control and Prevention (CDC).** 2007. Acute respiratory disease
943 associated with adenovirus serotype 14--four states, 2006–2007. *MMWR Morb. Mortal. Wkly. Rep.*
944 **56**:1181–1184.
- 945 17. **Louie JK, Kajon AE, Holodniy M, Guardia-LaBar L, Lee B, Petru AM, Hacker JK, Schnurr**
946 **DP.** 2008. Severe pneumonia due to adenovirus serotype 14: a new respiratory threat? *Clin.*
947 *Infect. Dis.* **46**:421–425.
- 948 18. **Lam E, Ramke M, Warnecke G, Schrepfer S, Kopfnagel V, Dobner T, Heim A.** 2015. Effective
949 apical infection of differentiated human bronchial epithelial cells and induction of proinflammatory
950 chemokines by the highly pneumotropic human adenovirus type 14p1. *PLoS One* **10**:e0131201.
- 951 19. **O’Flanagan D, O’Donnell J, Domegan L, Fitzpatrick F, Connell J, Coughlan S, De Gascun C,**
952 **Carr MJ.** 2011. First reported cases of human adenovirus serotype 14p1 infection, Ireland,
953 October 2009 to July 2010. *Euro Surveill.* **16**.
- 954 20. **Carr MJ, Kajon AE, Lu X, Dunford L, O’Reilly P, Holder P, De Gascun CF, Coughlan S,**

- 955 **Connell J, Erdman DD, Hall WW.** 2011. Deaths associated with human adenovirus-14p1
956 infections, Europe, 2009-2010. *Emerging Infect. Dis.* **17**:1402–1408.
- 957 21. **Benkő M, Harrach B, Kremer EJ.** 2014. Do nonhuman primate or bat adenoviruses pose a risk
958 for human health? *Future Microbiol* **9**:269–272.
- 959 22. **Chen EC, Yagi S, Kelly KR, Mendoza SP, Tarara RP, Canfield DR, Maninger N, Rosenthal A,**
960 **Spinner A, Bales KL, Schnurr DP, Lerche NW, Chiu CY.** 2011. Cross-species transmission of a
961 novel adenovirus associated with a fulminant pneumonia outbreak in a new world monkey colony.
962 *PLoS Pathog.* **7**:e1002155.
- 963 23. **To KKW, Tse H, Chan W-M, Choi GKY, Zhang AJX, Sridhar S, Wong SCY, Chan JFW, Chan**
964 **ASF, Woo PCY, Lau SKP, Lo JYC, Chan K-H, Cheng VCC, Yuen K-Y.** 2014. A novel psittacine
965 adenovirus identified during an outbreak of avian chlamydiosis and human psittacosis: zoonosis
966 associated with virus-bacterium coinfection in birds. *PLoS Negl. Trop. Dis.* **8**:e3318.
- 967 24. **Gray GC, McCarthy T, Lebeck MG, Schnurr DP, Russell KL, Kajon AE, Landry ML, Leland**
968 **DS, Storch GA, Ginocchio CC, Robinson CC, Demmler GJ, Saubolle MA, Kehl SC,**
969 **Selvarangan R, Miller MB, Chappell JD, Zerr DM, Kiska DL, Halstead DC, Capuano AW,**
970 **Setterquist SF, Chorazy ML, Dawson JD, Erdman DD.** 2007. Genotype prevalence and risk
971 factors for severe clinical adenovirus infection, United States 2004-2006. *Clin. Infect. Dis.*
972 **45**:1120–1131.
- 973 25. **Metzgar D, Osuna M, Kajon AE, Hawksworth AW, Irvine M, Russell KL.** 2007. Abrupt
974 emergence of diverse species B adenoviruses at US military recruit training centers. *J. Infect. Dis.*
975 **196**:1465–1473.
- 976 26. **Haque E, Banik U, Monowar T, Anthony L, Adhikary AK.** 2018. Worldwide increased
977 prevalence of human adenovirus type 3 (HAdV-3) respiratory infections is well correlated with
978 heterogeneous hypervariable regions (HVRs) of hexon. *PLoS One* **13**:e0194516.
- 979 27. **Ginn SL, Amaya AK, Alexander IE, Edelstein M, Abedi MR.** 2018. Gene therapy clinical trials
980 worldwide to 2017: An update. *J Gene Med* **20**:e3015.
- 981 28. **Jiang H, Gomez-Manzano C, Rivera-Molina Y, Lang FF, Conrad CA, Fueyo J.** 2015. Oncolytic
982 adenovirus research evolution: from cell-cycle checkpoints to immune checkpoints. *Curr Opin*
983 *Viro* **13**:33–39.
- 984 29. **Lawler SE, Speranza M-C, Cho C-F, Chiocca EA.** 2017. Oncolytic viruses in cancer treatment:
985 A review. *JAMA Oncol.* **3**:841–849.
- 986 30. **Hiwarkar P, Amrolia P, Sivaprakasam P, Lum SH, Doss H, O'Rafferty C, Petterson T, Patrick**
987 **K, Silva J, Slatter M, Lawson S, Rao K, Steward C, Gassas A, Veys P, Wynn R, United**
988 **Kingdom Paediatric Bone Marrow Transplant Group.** 2017. Brincidofovir is highly efficacious
989 in controlling adenoviremia in pediatric recipients of hematopoietic cell transplant. *Blood*
990 **129**:2033–2037.
- 991 31. **Wold WSM, Tollefson AE, Ying B, Spencer JF, Toth K.** 2019. Drug development against
992 human adenoviruses and its advancement by Syrian hamster models. *FEMS Microbiol. Rev.*
993 **43**:380–388.
- 994 32. **Reddy VS, Natchiar SK, Stewart PL, Nemerow GR.** 2010. Crystal structure of human
995 adenovirus at 3.5 Å resolution. *Science* **329**:1071–1075.
- 996 33. **Benevento M, Di Palma S, Snijder J, Moyer CL, Reddy VS, Nemerow GR, Heck AJR.** 2014.
997 Adenovirus composition, proteolysis, and disassembly studied by in-depth qualitative and
998 quantitative proteomics. *J. Biol. Chem.* **289**:11421–11430.
- 999 34. **Flatt JW, Greber UF.** 2017. Viral mechanisms for docking and delivering at nuclear pore
1000 complexes. *Semin. Cell Dev. Biol.* **68**:59–71.
- 1001 35. **Liu H, Jin L, Koh SBS, Atanasov I, Schein S, Wu L, Zhou ZH.** 2010. Atomic structure of human
1002 adenovirus by cryo-EM reveals interactions among protein networks. *Science* **329**:1038–1043.
- 1003 36. **Greber UF, Flatt JW.** 2019. Adenovirus entry: from infection to immunity. *Annu. Rev. Virol.*
1004 **6**:177–197.
- 1005 37. **Wang I-H, Burckhardt CJ, Yakimovich A, Greber UF.** 2018. Imaging, Tracking and

- 1006 Computational Analyses of Virus Entry and Egress with the Cytoskeleton. *Viruses* **10**.
1007 38. **Suomalainen M, Greber UF**. 2013. Uncoating of non-enveloped viruses. *Curr Opin Virol* **3**:27–
1008 33.
1009 39. **Greber UF**. 2002. Signalling in viral entry. *Cellular and Molecular Life Sciences (CMLS)* **59**:608–
1010 626.
1011 40. **Sohn S-Y, Hearing P**. 2019. Adenoviral strategies to overcome innate cellular responses to
1012 infection. *FEBS Lett*. **593**:3484–3495.
1013 41. **Prusinkiewicz MA, Mymryk JS**. 2019. Metabolic reprogramming of the host cell by human
1014 adenovirus infection. *Viruses* **11**.
1015 42. **Pied N, Wodrich H**. 2019. Imaging the adenovirus infection cycle. *FEBS Lett*. **593**:3419–3448.
1016 43. **Oliveira ERA, Bouvier M**. 2019. Immune evasion by adenoviruses: a window into host-virus
1017 adaptation. *FEBS Lett*. **593**:3496–3503.
1018 44. **Nemerow G, Flint J**. 2019. Lessons learned from adenovirus (1970-2019). *FEBS Lett*. **593**:3395–
1019 3418.
1020 45. **Lynch KL, Gooding LR, Garnett-Benson C, Ornelles DA, Avgousti DC**. 2019. Epigenetics and
1021 the dynamics of chromatin during adenovirus infections. *FEBS Lett*. **593**:3551–3570.
1022 46. **Kleinberger T**. 2019. Biology of the adenovirus E4orf4 protein: from virus infection to cancer cell
1023 death. *FEBS Lett*.
1024 47. **Hidalgo P, Ip WH, Dobner T, Gonzalez RA**. 2019. The biology of the adenovirus E1B 55K
1025 protein. *FEBS Lett*. **593**:3504–3517.
1026 48. **Charman M, Herrmann C, Weitzman MD**. 2019. Viral and cellular interactions during adenovirus
1027 DNA replication. *FEBS Lett*. **593**:3531–3550.
1028 49. **Atasheva S, Yao J, Shayakhmetov DM**. 2019. Innate immunity to adenovirus: lessons from
1029 mice. *FEBS Lett*. **593**:3461–3483.
1030 50. **Allen RJ, Byrnes AP**. 2019. Interaction of adenovirus with antibodies, complement, and
1031 coagulation factors. *FEBS Lett*. **593**:3449–3460.
1032 51. **Burckhardt CJ, Suomalainen M, Schoenenberger P, Boucke K, Hemmi S, Greber UF**. 2011.
1033 Drifting motions of the adenovirus receptor CAR and immobile integrins initiate virus uncoating
1034 and membrane lytic protein exposure. *Cell Host Microbe* **10**:105–117.
1035 52. **Meier O, Greber UF**. 2004. Adenovirus endocytosis. *J Gene Med* **6 Suppl 1**:S152-63.
1036 53. **Gastaldelli M, Imelli N, Boucke K, Amstutz B, Meier O, Greber UF**. 2008. Infectious
1037 adenovirus type 2 transport through early but not late endosomes. *Traffic* **9**:2265–2278.
1038 54. **Wolfrum N, Greber UF**. 2013. Adenovirus signalling in entry. *Cell Microbiol*. **15**:53–62.
1039 55. **Luisoni S, Suomalainen M, Boucke K, Tanner LB, Wenk MR, Guan XL, Grzybek M, Coskun
1040 Ü, Greber UF**. 2015. Co-option of Membrane Wounding Enables Virus Penetration into Cells. *Cell
1041 Host Microbe* **18**:75–85.
1042 56. **Wiethoff CM, Wodrich H, Gerace L, Nemerow GR**. 2005. Adenovirus protein VI mediates
1043 membrane disruption following capsid disassembly. *J. Virol*. **79**:1992–2000.
1044 57. **Leopold PL, Kreitzer G, Miyazawa N, Rempel S, Pfister KK, Rodriguez-Boulan E, Crystal
1045 RG**. 2000. Dynein- and microtubule-mediated translocation of adenovirus serotype 5 occurs after
1046 endosomal lysis. *Hum. Gene Ther*. **11**:151–165.
1047 58. **Suomalainen M, Nakano MY, Keller S, Boucke K, Stidwill RP, Greber UF**. 1999. Microtubule-
1048 dependent plus- and minus end-directed motilities are competing processes for nuclear targeting
1049 of adenovirus. *J. Cell Biol*. **144**:657–672.
1050 59. **Wang I-H, Burckhardt CJ, Yakimovich A, Morf MK, Greber UF**. 2017. The nuclear export factor
1051 CRM1 controls juxta-nuclear microtubule-dependent virus transport. *J. Cell Sci*. **130**:2185–2195.
1052 60. **Zhou J, Scherer J, Yi J, Vallee RB**. 2018. Role of kinesins in directed adenovirus transport and
1053 cytoplasmic exploration. *PLoS Pathog*. **14**:e1007055.
1054 61. **Bremner KH, Scherer J, Yi J, Vershinin M, Gross SP, Vallee RB**. 2009. Adenovirus transport
1055 via direct interaction of cytoplasmic dynein with the viral capsid hexon subunit. *Cell Host Microbe*

- 1056 6:523–535.
- 1057 62. **Bauer M, Flatt JW, Seiler D, Cardel B, Emmenlauer M, Boucke K, Suomalainen M, Hemmi S,**
1058 **Greber UF.** 2019. The e3 ubiquitin ligase mind bomb 1 controls adenovirus genome release at
1059 the nuclear pore complex. *Cell Rep.* **29**:3785–3795.e8.
- 1060 63. **Pelka P, Ablack JNG, Fonseca GJ, Yousef AF, Mymryk JS.** 2008. Intrinsic structural disorder
1061 in adenovirus E1A: a viral molecular hub linking multiple diverse processes. *J. Virol.* **82**:7252–
1062 7263.
- 1063 64. **King CR, Zhang A, Tessier TM, Gameiro SF, Mymryk JS.** 2018. Hacking the cell: network
1064 intrusion and exploitation by adenovirus E1A. *MBio* **9**.
- 1065 65. **Berk AJ.** 2005. Recent lessons in gene expression, cell cycle control, and cell biology from
1066 adenovirus. *Oncogene* **24**:7673–7685.
- 1067 66. **Ferrari R, Pellegrini M, Horwitz GA, Xie W, Berk AJ, Kurdistani SK.** 2008. Epigenetic
1068 reprogramming by adenovirus e1a. *Science* **321**:1086–1088.
- 1069 67. **Rao L, Debbas M, Sabbatini P, Hockenbery D, Korsmeyer S, White E.** 1992. The adenovirus
1070 E1A proteins induce apoptosis, which is inhibited by the E1B 19-kDa and Bcl-2 proteins. *Proc.*
1071 *Natl. Acad. Sci. USA* **89**:7742–7746.
- 1072 68. **Zemke NR, Berk AJ.** 2017. The adenovirus E1A C terminus suppresses a delayed antiviral
1073 response and modulates RAS signaling. *Cell Host Microbe* **22**:789–800.e5.
- 1074 69. **Greber UF.** 1998. Virus assembly and disassembly: the adenovirus cysteine protease as a trigger
1075 factor. *Rev Med Virol* **8**:213–222.
- 1076 70. **Mangel WF, San Martín C.** 2014. Structure, function and dynamics in adenovirus maturation.
1077 *Viruses* **6**:4536–4570.
- 1078 71. **Ahi YS, Mittal SK.** 2016. Components of adenovirus genome packaging. *Front. Microbiol.*
1079 **7**:1503.
- 1080 72. **Yakimovich A, Gumpert H, Burckhardt CJ, Lütschg VA, Jurgeit A, Sbalzarini IF, Greber UF.**
1081 2012. Cell-free transmission of human adenovirus by passive mass transfer in cell culture
1082 simulated in a computer model. *J. Virol.* **86**:10123–10137.
- 1083 73. **Tollefson AE, Scaria A, Hermiston TW, Ryerse JS, Wold LJ, Wold WS.** 1996. The adenovirus
1084 death protein (E3-11.6K) is required at very late stages of infection for efficient cell lysis and
1085 release of adenovirus from infected cells. *J. Virol.* **70**:2296–2306.
- 1086 74. **Doronin K, Toth K, Kuppaswamy M, Krajcsi P, Tollefson AE, Wold WSM.** 2003.
1087 Overexpression of the ADP (E3-11.6K) protein increases cell lysis and spread of adenovirus.
1088 *Virology* **305**:378–387.
- 1089 75. **Haisma HJ, Kamps JAAM, Kamps GK, Plantinga JA, Rots MG, Bellu AR.** 2008. Polyinosinic
1090 acid enhances delivery of adenovirus vectors in vivo by preventing sequestration in liver
1091 macrophages. *J. Gen. Virol.* **89**:1097–1105.
- 1092 76. **Kajon AE, Gigliotti AP, Harrod KS.** 2003. Acute inflammatory response and remodeling of
1093 airway epithelium after subspecies B1 human adenovirus infection of the mouse lower respiratory
1094 tract. *J. Med. Virol.* **71**:233–244.
- 1095 77. **Beerli C, Yakimovich A, Kilcher S, Reynoso GV, Fläschner G, Müller DJ, Hickman HD,**
1096 **Mercer J.** 2019. Vaccinia virus hijacks EGFR signalling to enhance virus spread through rapid
1097 and directed infected cell motility. *Nat. Microbiol.* **4**:216–225.
- 1098 78. **Roberts KL, Smith GL.** 2008. Vaccinia virus morphogenesis and dissemination. *Trends*
1099 *Microbiol.* **16**:472–479.
- 1100 79. **Doceul V, Hollinshead M, van der Linden L, Smith GL.** 2010. Repulsion of superinfecting
1101 virions: a mechanism for rapid virus spread. *Science* **327**:873–876.
- 1102 80. **Zhong P, Agosto LM, Munro JB, Mothes W.** 2013. Cell-to-cell transmission of viruses. *Curr*
1103 *Opin Virol* **3**:44–50.
- 1104 81. **Mothes W, Sherer NM, Jin J, Zhong P.** 2010. Virus cell-to-cell transmission. *J. Virol.* **84**:8360–
1105 8368.

- 1106 82. **Burckhardt CJ, Greber UF.** 2009. Virus movements on the plasma membrane support infection
1107 and transmission between cells. *PLoS Pathog.* **5**:e1000621.
- 1108 83. **van der Grein SG, Defourny KAY, Slot EFJ, Nolte-'t Hoen ENM.** 2018. Intricate relationships
1109 between naked viruses and extracellular vesicles in the crosstalk between pathogen and host.
1110 *Semin Immunopathol* **40**:491–504.
- 1111 84. **Jansens RJJ, Tishchenko A, Favoreel HW.** 2020. Bridging the Gap: Virus Long-Distance
1112 Spread via Tunneling Nanotubes. *J. Virol.* **94**.
- 1113 85. **Danthi P.** 2016. Viruses and the diversity of cell death. *Annu. Rev. Virol.* **3**:533–553.
- 1114 86. **Madan V, Castelló A, Carrasco L.** 2008. Viroporins from RNA viruses induce caspase-
1115 dependent apoptosis. *Cell Microbiol.* **10**:437–451.
- 1116 87. **Scott C, Griffin S.** 2015. Viroporins: structure, function and potential as antiviral targets. *J. Gen.*
1117 *Virol.* **96**:2000–2027.
- 1118 88. **Zhang Y, Chen X, Gueydan C, Han J.** 2018. Plasma membrane changes during programmed
1119 cell deaths. *Cell Res.* **28**:9–21.
- 1120 89. **Dhingra A, Hage E, Ganzenmueller T, Böttcher S, Hofmann J, Hamprecht K, Obermeier P,**
1121 **Rath B, Hausmann F, Dobner T, Heim A.** 2019. Molecular evolution of human adenovirus
1122 (hadv) species C. *Sci. Rep.* **9**:1039.
- 1123 90. **Robinson CM, Singh G, Lee JY, Dehghan S, Rajaiya J, Liu EB, Yousuf MA, Betensky RA,**
1124 **Jones MS, Dyer DW, Seto D, Chodosh J.** 2013. Molecular evolution of human adenoviruses.
1125 *Sci. Rep.* **3**:1812.
- 1126 91. **Davison AJ, Akter P, Cunningham C, Dolan A, Addison C, Dargan DJ, Hassan-Walker AF,**
1127 **Emery VC, Griffiths PD, Wilkinson GWG.** 2003. Homology between the human cytomegalovirus
1128 RL11 gene family and human adenovirus E3 genes. *J. Gen. Virol.* **84**:657–663.
- 1129 92. **Scaria A, Tollefson AE, Saha SK, Wold WS.** 1992. The E3-11.6K protein of adenovirus is an
1130 Asn-glycosylated integral membrane protein that localizes to the nuclear membrane. *Virology*
1131 **191**:743–753.
- 1132 93. **Georgi F, Greber UF.** 2020. The Adenovirus Death Protein - A small membrane protein controls
1133 cell lysis and disease. *FEBS Lett.* DOI: 10.1002/1873-3468.13848
- 1134 94. **Tollefson AE, Scaria A, Saha SK, Wold WS.** 1992. The 11,600-MW protein encoded by region
1135 E3 of adenovirus is expressed early but is greatly amplified at late stages of infection. *J. Virol.*
1136 **66**:3633–3642.
- 1137 95. **Wold WS, Cladaras C, Magie SC, Yacoub N.** 1984. Mapping a new gene that encodes an
1138 11,600-molecular-weight protein in the E3 transcription unit of adenovirus 2. *J. Virol.* **52**:307–313.
- 1139 96. **Ito H, Aoki H, Kühnel F, Kondo Y, Kubicka S, Wirth T, Iwado E, Iwamaru A, Fujiwara K, Hess**
1140 **KR, Lang FF, Sawaya R, Kondo S.** 2006. Autophagic cell death of malignant glioma cells
1141 induced by a conditionally replicating adenovirus. *J. Natl. Cancer Inst.* **98**:625–636.
- 1142 97. **Jiang H, Gomez-Manzano C, Aoki H, Alonso MM, Kondo S, McCormick F, Xu J, Kondo Y,**
1143 **Bekele BN, Colman H, Lang FF, Fueyo J.** 2007. Examination of the therapeutic potential of
1144 Delta-24-RGD in brain tumor stem cells: role of autophagic cell death. *J. Natl. Cancer Inst.*
1145 **99**:1410–1414.
- 1146 98. **Jiang H, White EJ, Ríos-Vicil CI, Xu J, Gomez-Manzano C, Fueyo J.** 2011. Human adenovirus
1147 type 5 induces cell lysis through autophagy and autophagy-triggered caspase activity. *J. Virol.*
1148 **85**:4720–4729.
- 1149 99. **Abou El Hassan MAI, van der Meulen-Muileman I, Abbas S, Kruyt FAE.** 2004. Conditionally
1150 replicating adenoviruses kill tumor cells via a basic apoptotic machinery-independent mechanism
1151 that resembles necrosis-like programmed cell death. *J. Virol.* **78**:12243–12251.
- 1152 100. **Georgi F, Kuttler F, Murer L, Andriasyan V, Witte R, Yakimovich A, Turcatti G, Greber UF.**
1153 2020. High-content image-based drug screen identifies a clinical compound against cell
1154 transmission of adenovirus. *BioRxiv.* DOI: <https://doi.org/10.1101/2020.05.15.098061>
- 1155 101. **Yakimovich A, Andriasyan V, Witte R, Wang I-H, Prasad V, Suomalainen M, Greber UF.**
1156 2015. Plaque2.0-A High-Throughput Analysis Framework to Score Virus-Cell Transmission and

- 1157 Clonal Cell Expansion. *PLoS One* **10**:e0138760.
- 1158 102. **Kaldor SW, Kalish VJ, Davies JF, Shetty BV, Fritz JE, Appelt K, Burgess JA, Campanale**
1159 **KM, Chirgadze NY, Clawson DK, Dressman BA, Hatch SD, Khalil DA, Kosa MB,**
1160 **Lubbehusen PP, Muesing MA, Patick AK, Reich SH, Su KS, Tatlock JH.** 1997. Viracept
1161 (nelfinavir mesylate, AG1343): a potent, orally bioavailable inhibitor of HIV-1 protease. *J. Med.*
1162 *Chem.* **40**:3979–3985.
- 1163 103. **Greber UF, Willetts M, Webster P, Helenius A.** 1993. Stepwise dismantling of adenovirus 2
1164 during entry into cells. *Cell* **75**:477–486.
- 1165 104. **Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG.** 2005. Simple and highly
1166 efficient BAC recombineering using galK selection. *Nucleic Acids Res.* **33**:e36.
- 1167 105. **Sirena D, Lilienfeld B, Eisenhut M, Kälin S, Boucke K, Beerli RR, Vogt L, Ruedl C,**
1168 **Bachmann MF, Greber UF, Hemmi S.** 2004. The human membrane cofactor CD46 is a receptor
1169 for species B adenovirus serotype 3. *J. Virol.* **78**:4454–4462.
- 1170 106. **Hemmi S, Geertsens R, Mezzacasa A, Peter I, Dummer R.** 1998. The presence of human
1171 coxsackievirus and adenovirus receptor is associated with efficient adenovirus-mediated
1172 transgene expression in human melanoma cell cultures. *Hum. Gene Ther.* **9**:2363–2373.
- 1173 107. **Tollefson AE, Scaria A, Ying B, Wold WSM.** 2003. Mutations within the ADP (E3-11.6K) protein
1174 alter processing and localization of ADP and the kinetics of cell lysis of adenovirus-infected cells.
1175 *J. Virol.* **77**:7764–7778.
- 1176 108. **Adrian T, Wadell G, Hierholzer JC, Wigand R.** 1986. DNA restriction analysis of adenovirus
1177 prototypes 1 to 41. *Arch. Virol.* **91**:277–290.
- 1178 109. **Pacesa M, Hendrickx R, Bieri M, Flatt JW, Greber UF, Hemmi S.** 2017. Small-size recombinant
1179 adenoviral hexon protein fragments for the production of virus-type specific antibodies. *Virol. J.*
1180 **14**:158.
- 1181 110. **Kajon AE, Hang J, Hawksworth A, Metzgar D, Hage E, Hansen CJ, Kuschner RA, Blair P,**
1182 **Russell KL, Jarman RG.** 2015. Molecular Epidemiology of Adenovirus Type 21 Respiratory
1183 Strains Isolated From US Military Trainees (1996-2014). *J. Infect. Dis.* **212**:871–880.
- 1184 111. **Robinson M, Li B, Ge Y, Ko D, Yendluri S, Harding T, VanRoey M, Spindler KR, Jooss K.**
1185 2009. Novel immunocompetent murine tumor model for evaluation of conditionally replication-
1186 competent (oncolytic) murine adenoviral vectors. *J. Virol.* **83**:3450–3462.
- 1187 112. **Studer L.** 2017. Overcome the species barrier of productive human adenovirus infection in
1188 murine cells. Master thesis, Department of Molecular Life Sciences, University of Zurich.
- 1189 113. **Jetzer T.** 2018. Characterization of the human/mouse adenovirus productive replication barrier by
1190 transcriptionally targeting genes of type B and C adenoviruses. Master thesis, Department of
1191 Molecular Life Sciences, University of Zurich.
- 1192 114. **Wold WSM, Deutscher SL, Takemori N, Bhat BM, Magie SC.** 1986. Evidence that AGAUA and
1193 CCAGA initiate translation in the same mRNA in region E3 of adenovirus. *Virology* **148**:168–180.
- 1194 115. **Bhat BM, Wold WS.** 1986. Adenovirus mutants with splice-enhancing mutations in the E3
1195 complex transcription unit are also defective in E3A RNA 3'-end formation. *J. Virol.* **57**:1155–
1196 1158.
- 1197 116. **Wold WS, Gooding LR.** 1991. Region E3 of adenovirus: a cassette of genes involved in host
1198 immunosurveillance and virus-cell interactions. *Virology* **184**:1–8.
- 1199 117. **Hendrickx RC.** 2016. Study on Mouse Adenovirus Biology and Development of Their Vectors.
1200 Doctoral dissertation, Department of Molecular Life Sciences, University of Zurich.
- 1201 118. **Bieri M.** 2018. Development of Adenovirus Reporter Viruses and Studies on Mouse Adenovirus-2
1202 and -3 Receptor Candidates. Doctoral dissertation, Department of Molecular Life Sciences,
1203 University of Zurich.
- 1204 119. **Glauser DL, Seyffert M, Strasser R, Franchini M, Laimbacher AS, Dresch C, de Oliveira AP,**
1205 **Vogel R, Büning H, Salvetti A, Ackermann M, Fraefel C.** 2010. Inhibition of herpes simplex
1206 virus type 1 replication by adeno-associated virus rep proteins depends on their combined DNA-
1207 binding and ATPase/helicase activities. *J. Virol.* **84**:3808–3824.

- 1208 120. **Ali A, Roossinck MJ.** 2007. Rapid and efficient purification of Cowpea chlorotic mottle virus by
1209 sucrose cushion ultracentrifugation. *J. Virol. Methods* **141**:84–86.
- 1210 121. **Crameri M, Bauer M, Caduff N, Walker R, Steiner F, Franzoso FD, Gujer C, Boucke K,**
1211 **Kucera T, Zbinden A, Münz C, Fraefel C, Greber UF, Pavlovic J.** 2018. MxB is an interferon-
1212 induced restriction factor of human herpesviruses. *Nat. Commun.* **9**:1980.
- 1213 122. **Prasad V, Suomalainen M, Pennauer M, Yakimovich A, Andriasyan V, Hemmi S, Greber UF.**
1214 2014. Chemical induction of unfolded protein response enhances cancer cell killing through lytic
1215 virus infection. *J. Virol.* **88**:13086–13098.
- 1216 123. **Prasad V, Suomalainen M, Jasiqi Y, Hemmi S, Hearing P, Hosie L, Burgert H-G, Greber UF.**
1217 2020. The UPR sensor IRE1 α and the adenovirus E3-19K glycoprotein sustain persistent and lytic
1218 infections. *Nat. Commun.* **11**:1997.
- 1219 124. **Spiegel M.** 2009. Real-time and Dynamic Monitoring of Virus-mediated Cytopathogenicity.
1220 *Biochemica* 15–17.
- 1221 125. **Carpenter AE, Jones TR, Lamprecht MR, Clarke C, Kang IH, Friman O, Guertin DA, Chang**
1222 **JH, Lindquist RA, Moffat J, Golland P, Sabatini DM.** 2006. CellProfiler: image analysis software
1223 for identifying and quantifying cell phenotypes. *Genome Biol.* **7**:R100.
- 1224 126. **R Core Team.** 2018. R: A Language and Environment for Statistical Computing. Computer
1225 software, R Foundation for Statistical Computing, Vienna, Austria.
- 1226 127. **Greber UF, Suomalainen M, Stidwill RP, Boucke K, Ebersold MW, Helenius A.** 1997. The role
1227 of the nuclear pore complex in adenovirus DNA entry. *EMBO J.* **16**:5998–6007.
- 1228 128. **Baum SG, Horwitz MS, Maizel JV.** 1972. Studies of the mechanism of enhancement of human
1229 adenovirus infection in monkey cells by simian virus 40. *J. Virol.* **10**:211–219.
- 1230 129. **Varghese R, Mityas Y, Stewart PL, Ralston R.** 2004. Postentry neutralization of adenovirus
1231 type 5 by an antihexon antibody. *J. Virol.* **78**:12320–12332.
- 1232 130. **Hausl MA, Zhang W, Muther N, Rauschhuber C, Franck HG, Merricks EP, Nichols TC, Kay**
1233 **MA, Ehrhardt A.** 2010. Hyperactive sleeping beauty transposase enables persistent phenotypic
1234 correction in mice and a canine model for hemophilia B. *Mol. Ther.* **18**:1896–1906.
- 1235 131. **Topal MD.** 1984. Molecular mechanisms of chemical mutagenesis: 9-aminoacridine inhibits DNA
1236 replication in vitro by destabilizing the DNA growing point and interacting with the DNA
1237 polymerase. *Biochemistry* **23**:2367–2372.
- 1238 132. **Moyle GJ, Youle M, Higgs C, Monaghan J, Prince W, Chapman S, Clendeninn N, Nelson**
1239 **MR.** 1998. Safety, pharmacokinetics, and antiretroviral activity of the potent, specific human
1240 immunodeficiency virus protease inhibitor nelfinavir: results of a phase I/II trial and extended
1241 follow-up in patients infected with human immunodeficiency virus. *J. Clin. Pharmacol.* **38**:736–
1242 743.
- 1243 133. **Gantt S, Carlsson J, Ikoma M, Gachelet E, Gray M, Geballe AP, Corey L, Casper C, Lagunoff**
1244 **M, Vieira J.** 2011. The HIV protease inhibitor nelfinavir inhibits Kaposi's sarcoma-associated
1245 herpesvirus replication in vitro. *Antimicrob. Agents Chemother.* **55**:2696–2703.
- 1246 134. **Imelli N, Ruzsics Z, Puntener D, Gastaldelli M, Greber UF.** 2009. Genetic reconstitution of the
1247 human adenovirus type 2 temperature-sensitive 1 mutant defective in endosomal escape. *Virol. J.*
1248 **6**:174.
- 1249 135. **Tollefson AE, Ryerse JS, Scaria A, Hermiston TW, Wold WS.** 1996. The E3-11.6-kDa
1250 adenovirus death protein (ADP) is required for efficient cell death: characterization of cells
1251 infected with adp mutants. *Virology* **220**:152–162.
- 1252 136. **Deutscher SL, Bhat BM, Pursley MH, Cladaras C, Wold WS.** 1985. Novel deletion mutants that
1253 enhance a distant upstream 5' splice in the E3 transcription unit of adenovirus 2. *Nucleic Acids*
1254 *Res.* **13**:5771–5788.
- 1255 137. **Reily C, Stewart TJ, Renfrow MB, Novak J.** 2019. Glycosylation in health and disease. *Nat.*
1256 *Rev. Nephrol.* **15**:346–366.
- 1257 138. **Kalu NN, Desai PJ, Shirley CM, Gibson W, Dennis PA, Ambinder RF.** 2014. Nelfinavir inhibits
1258 maturation and export of herpes simplex virus 1. *J. Virol.* **88**:5455–5461.

- 1259 139. **Gantt S, Gachelet E, Carlsson J, Barcy S, Casper C, Lagunoff M.** 2015. Nelfinavir impairs
1260 glycosylation of herpes simplex virus 1 envelope proteins and blocks virus maturation. *Adv Virol*
1261 **2015**:687162.
- 1262 140. **Yamamoto N, Yang R, Yoshinaka Y, Amari S, Nakano T, Cinatl J, Rabenau H, Doerr HW,**
1263 **Hunsmann G, Otaka A, Tamamura H, Fujii N, Yamamoto N.** 2004. HIV protease inhibitor
1264 nelfinavir inhibits replication of SARS-associated coronavirus. *Biochem. Biophys. Res. Commun.*
1265 **318**:719–725.
- 1266 141. **Toma S, Yamashiro T, Arakaki S, Shiroma J, Maeshiro T, Hibiya K, Sakamoto N, Kinjo F,**
1267 **Tateyama M, Fujita J.** 2009. Inhibition of intracellular hepatitis C virus replication by nelfinavir
1268 and synergistic effect with interferon-alpha. *J Viral Hepat* **16**:506–512.
- 1269 142. **Johnson DC, Spear PG.** 1983. O-linked oligosaccharides are acquired by herpes simplex virus
1270 glycoproteins in the Golgi apparatus. *Cell* **32**:987–997.
- 1271 143. **Guan M, Fousek K, Jiang C, Guo S, Synold T, Xi B, Shih C-C, Chow WA.** 2011. Nelfinavir
1272 induces liposarcoma apoptosis through inhibition of regulated intramembrane proteolysis of
1273 SREBP-1 and ATF6. *Clin. Cancer Res.* **17**:1796–1806.
- 1274 144. **Guan M, Fousek K, Chow WA.** 2012. Nelfinavir inhibits regulated intramembrane proteolysis of
1275 sterol regulatory element binding protein-1 and activating transcription factor 6 in castration-
1276 resistant prostate cancer. *FEBS J.* **279**:2399–2411.
- 1277 145. **Guan M, Su L, Yuan Y-C, Li H, Chow WA.** 2015. Nelfinavir and nelfinavir analogs block site-2
1278 protease cleavage to inhibit castration-resistant prostate cancer. *Sci. Rep.* **5**:9698.
- 1279 146. **Chow WA, Guo S, Valdes-Albini F.** 2006. Nelfinavir induces liposarcoma apoptosis and cell
1280 cycle arrest by upregulating sterol regulatory element binding protein-1. *Anticancer Drugs* **17**:891–
1281 903.
- 1282 147. **Caron M, Auclair M, Sterlingot H, Kornprobst M, Capeau J.** 2003. Some HIV protease
1283 inhibitors alter lamin A/C maturation and stability, SREBP-1 nuclear localization and adipocyte
1284 differentiation. *AIDS* **17**:2437–2444.
- 1285 148. **Yang Y, Ikezoe T, Takeuchi T, Adachi Y, Ohtsuki Y, Takeuchi S, Koeffler HP, Taguchi H.**
1286 **2005.** HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer
1287 LNCaP cells in vitro and in vivo in conjunction with blockade of androgen receptor STAT3 and
1288 AKT signaling. *Cancer Sci.* **96**:425–433.
- 1289 149. **Gills JJ, Lopiccio J, Tsurutani J, Shoemaker RH, Best CJM, Abu-Asab MS, Borojerdi J,**
1290 **Warfel NA, Gardner ER, Danish M, Hollander MC, Kawabata S, Tsokos M, Figg WD, Steeg**
1291 **PS, Dennis PA.** 2007. Nelfinavir, A lead HIV protease inhibitor, is a broad-spectrum, anticancer
1292 agent that induces endoplasmic reticulum stress, autophagy, and apoptosis in vitro and in vivo.
1293 *Clin. Cancer Res.* **13**:5183–5194.
- 1294 150. **Koltai T.** 2015. Nelfinavir and other protease inhibitors in cancer: mechanisms involved in
1295 anticancer activity. [version 2; peer review: 2 approved]. *F1000Res.* **4**:9.
- 1296 151. **Brüning A, Gingelmaier A, Friese K, Mylonas I.** 2010. New prospects for nelfinavir in non-HIV-
1297 related diseases. *Curr Mol Pharmacol* **3**:91–97.
- 1298 152. **Shim JS, Liu JO.** 2014. Recent advances in drug repositioning for the discovery of new
1299 anticancer drugs. *Int. J. Biol. Sci.* **10**:654–663.
- 1300 153. **Bernstein WB, Dennis PA.** 2008. Repositioning HIV protease inhibitors as cancer therapeutics.
1301 *Curr Opin HIV AIDS* **3**:666–675.
- 1302 154. **Chow WA, Jiang C, Guan M.** 2009. Anti-HIV drugs for cancer therapeutics: back to the future?
1303 *Lancet Oncol.* **10**:61–71.
- 1304 155. **Gantt S, Casper C, Ambinder RF.** 2013. Insights into the broad cellular effects of nelfinavir and
1305 the HIV protease inhibitors supporting their role in cancer treatment and prevention. *Curr Opin*
1306 *Oncol* **25**:495–502.
- 1307 156. **Tollefson AE, Ying B, Spencer JF, Sagartz JE, Wold WSM, Toth K.** 2017. Pathology in
1308 Permissive Syrian Hamsters after Infection with Species C Human Adenovirus (HAdV-C) Is the
1309 Result of Virus Replication: HAdV-C6 Replicates More and Causes More Pathology than HAdV-

- 1310 C5. *J. Virol.* **91**.
- 1311 157. **Radke JR, Yong SL, Cook JL.** 2016. Low-Level Expression of the E1B 20-Kilodalton Protein by
1312 Adenovirus 14p1 Enhances Viral Immunopathogenesis. *J. Virol.* **90**:497–505.
- 1313 158. **Andriasyan V, Yakimovich A, Georgi F, Petkidis A, Witte R, Puntener D, Greber UF.** 2019.
1314 Deep learning of virus infections reveals mechanics of lytic cells. *BioRxiv*. DOI:
1315 <https://doi.org/10.1101/798074>
- 1316 159. **Zou A, Atencio I, Huang W-M, Horn M, Ramachandra M.** 2004. Overexpression of adenovirus
1317 E3-11.6K protein induces cell killing by both caspase-dependent and caspase-independent
1318 mechanisms. *Virology* **326**:240–249.
- 1319 160. **Yun C-O, Kim E, Koo T, Kim H, Lee Y, Kim J-H.** 2005. ADP-overexpressing adenovirus elicits
1320 enhanced cytopathic effect by induction of apoptosis. *Cancer Gene Ther* **12**:61–71.
- 1321 161. **Davison AJ, Benkő M, Harrach B.** 2003. Genetic content and evolution of adenoviruses. *J. Gen.*
1322 *Virol.* **84**:2895–2908.
- 1323 162. **Hausmann J, Ortmann D, Witt E, Veit M, Seidel W.** 1998. Adenovirus death protein, a
1324 transmembrane protein encoded in the E3 region, is palmitoylated at the cytoplasmic tail. *Virology*
1325 **244**:343–351.
- 1326 163. **Veit M.** 2012. Palmitoylation of virus proteins. *Biol. Cell* **104**:493–515.
- 1327 164. **Blaskovic S, Blanc M, van der Goot FG.** 2013. What does S-palmitoylation do to membrane
1328 proteins? *FEBS J.* **280**:2766–2774.
- 1329 165. **Longer M, Shetty B, Zamansky I, Tyle P.** 1995. Preformulation studies of a novel HIV protease
1330 inhibitor, AG1343. *J. Pharm. Sci.* **84**:1090–1093.
- 1331 166. **Tetko IV, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin VA, Radchenko**
1332 **EV, Zefirov NS, Makarenko AS, Tanchuk VY, Prokopenko VV.** 2005. Virtual computational
1333 chemistry laboratory--design and description. *J. Comput. Aided Mol. Des.* **19**:453–463.
- 1334 167. **Jurgeit A, McDowell R, Moese S, Meldrum E, Schwendener R, Greber UF.** 2012. Niclosamide
1335 is a proton carrier and targets acidic endosomes with broad antiviral effects. *PLoS Pathog.*
1336 **8**:e1002976.
- 1337 168. **Fonseca BD, Diering GH, Bidinosti MA, Dalal K, Alain T, Balgi AD, Forestieri R, Nodwell M,**
1338 **Rajadurai CV, Gunaratnam C, Tee AR, Duong F, Andersen RJ, Orlowski J, Numata M,**
1339 **Sonenberg N, Roberge M.** 2012. Structure-activity analysis of niclosamide reveals potential role
1340 for cytoplasmic pH in control of mammalian target of rapamycin complex 1 (mTORC1) signaling.
1341 *J. Biol. Chem.* **287**:17530–17545.
- 1342 169. **Baker AT, Greenshields-Watson A, Coughlan L, Davies JA, Uusi-Kerttula H, Cole DK,**
1343 **Rizkallah PJ, Parker AL.** 2019. Diversity within the adenovirus fiber knob hypervariable loops
1344 influences primary receptor interactions. *Nat. Commun.* **10**:741.
- 1345 170. **Uchino J, Curiel DT, Ugai H.** 2014. Species D human adenovirus type 9 exhibits better virus-
1346 spread ability for antitumor efficacy among alternative serotypes. *PLoS One* **9**:e87342.
- 1347 171. **Chen CY, Senac JS, Weaver EA, May SM, Jelinek DF, Greipp P, Witzig T, Barry MA.** 2011.
1348 Species D adenoviruses as oncolytics against B-cell cancers. *Clin. Cancer Res.* **17**:6712–6722.
- 1349 172. **Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L,**
1350 **Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, Antonov AV, Arama E, Baehrecke EH,**
1351 **Barlev NA, Bazan NG, Bernassola F, Bertrand MJM, Bianchi K, Blagosklonny MV, Blomgren**
1352 **K, Borner C, Boya P, Brenner C, Campanella M, Candi E, Carmona-Gutierrez D, Cecconi F,**
1353 **Chan FK-M, Chandel NS, Cheng EH, Chipuk JE, Cidlowski JA, Ciechanover A, Cohen GM,**
1354 **Conrad M, Cubillos-Ruiz JR, Czabotar PE, D'Angiolella V, Dawson TM, Dawson VL, De**
1355 **Laurenzi V, De Maria R, Debatin K-M, DeBerardinis RJ, Deshmukh M, Di Daniele N, Di**
1356 **Virgilio F, Dixit VM, Dixon SJ, Duckett CS, Dynlacht BD, El-Deiry WS, Elrod JW, Fimia GM,**
1357 **Fulda S, García-Sáez AJ, Garg AD, Garrido C, Gavathiotis E, Golstein P, Gottlieb E, Green**
1358 **DR, Greene LA, Gronemeyer H, Gross A, Hajnoczky G, Hardwick JM, Harris IS, Hengartner**
1359 **MO, Hetz C, Ichijo H, Jäättelä M, Joseph B, Jost PJ, Juin PP, Kaiser WJ, Karin M, Kaufmann**
1360 **T, Kepp O, Kimchi A, Kitsis RN, Klionsky DJ, Knight RA, Kumar S, Lee SW, Lemasters JJ,**
1361 **Levine B, Linkermann A, Lipton SA, Lockshin RA, López-Otín C, Lowe SW, Luedde T, Lugli**

- 1362 E, MacFarlane M, Madeo F, Malewicz M, Malorni W, Manic G, Marine J-C, Martin SJ,
1363 Martinou J-C, Medema JP, Mehlen P, Meier P, Melino S, Miao EA, Molkenkin JD, Moll UM,
1364 Muñoz-Pinedo C, Nagata S, Nuñez G, Oberst A, Oren M, Overholtzer M, Pagano M,
1365 Panaretakis T, Pasparakis M, Penninger JM, Pereira DM, Pervaiz S, Peter ME, Piacentini M,
1366 Pinton P, Prehn JHM, Puthalakath H, Rabinovich GA, Rehm M, Rizzuto R, Rodrigues CMP,
1367 Rubinsztein DC, Rudel T, Ryan KM, Sayan E, Scorrano L, Shao F, Shi Y, Silke J, Simon H-U,
1368 Sistigu A, Stockwell BR, Strasser A, Szabadkai G, Tait SWG, Tang D, Tavernarakis N,
1369 Thorburn A, Tsujimoto Y, Turk B, Vanden Berghe T, Vandenabeele P, Vander Heiden MG,
1370 Villunger A, Virgin HW, Vousden KH, Vucic D, Wagner EF, Walczak H, Wallach D, Wang Y,
1371 Wells JA, Wood W, Yuan J, Zakeri Z, Zhivotovsky B, Zitvogel L, Melino G, Kroemer G. 2018.
1372 Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell
1373 Death 2018. *Cell Death Differ.* **25**:486–541.
- 1374 173. Dziengelewski C, Rodrigue M-A, Caillier A, Jacquet K, Boulanger M-C, Bergeman J, Fuchs
1375 M, Lambert H, Laprise P, Richard DE, Bordeleau F, Huot M-É, Lavoie JN. 2020. Adenoviral
1376 protein E4orf4 interacts with the polarity protein Par3 to induce nuclear rupture and tumor cell
1377 death. *J. Cell Biol.* **219**.
- 1378 174. Verboon JM, Nakamura M, Davidson KA, Decker JR, Nandakumar V, Parkhurst SM. 2020.
1379 Wash and the wash regulatory complex function in nuclear envelope budding. *J Cell Sci*
1380 doi:10.1242/jcs.243576.
- 1381 175. Hagen C, Dent KC, Zeev-Ben-Mordehai T, Grange M, Bosse JB, Whittle C, Klupp BG,
1382 Siebert CA, Vasishtan D, Bäuerlein FJB, Cheleski J, Werner S, Guttman P, Rehbein S,
1383 Henzler K, Demmerle J, Adler B, Koszinowski U, Schermelleh L, Schneider G, Enquist LW,
1384 Plitzko JM, Mettenleiter TC, Grünwald K. 2015. Structural basis of vesicle formation at the
1385 inner nuclear membrane. *Cell* **163**:1692–1701.
- 1386 176. Lippincott-Schwartz J, Freed EO, van Engelenburg SB. 2017. A Consensus View of ESCRT-
1387 Mediated Human Immunodeficiency Virus Type 1 Abscission. *Annu. Rev. Virol.* **4**:309–325.
- 1388 177. Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L, Jeong S-H, Walker C, Lanford
1389 RE, Lemon SM. 2013. A pathogenic picornavirus acquires an envelope by hijacking cellular
1390 membranes. *Nature* **496**:367–371.
- 1391 178. Hurley JH. 2015. ESCRTs are everywhere. *EMBO J.* **34**:2398–2407.
- 1392 179. Murali VK, Ornelles DA, Gooding LR, Wilms HT, Huang W, Tollefson AE, Wold WSM,
1393 Garnett-Benson C. 2014. Adenovirus death protein (ADP) is required for lytic infection of human
1394 lymphocytes. *J. Virol.* **88**:903–912.
- 1395 180. Garnett CT, Erdman D, Xu W, Gooding LR. 2002. Prevalence and quantitation of species C
1396 adenovirus DNA in human mucosal lymphocytes. *J. Virol.* **76**:10608–10616.
- 1397 181. Zheng Y, Stamminger T, Hearing P. 2016. E2f/rb family proteins mediate interferon induced
1398 repression of adenovirus immediate early transcription to promote persistent viral infection. *PLoS*
1399 *Pathog.* **12**:e1005415.
- 1400 182. Kosulin K, Geiger E, Vécsei A, Huber WD, Rauch M, Brenner E, Wrba F, Hammer K,
1401 Innerhofer A, Pötschger U, Lawitschka A, Matthes-Leodolter S, Fritsch G, Lion T. 2016.
1402 Persistence and reactivation of human adenoviruses in the gastrointestinal tract. *Clin. Microbiol.*
1403 *Infect.* **22**:381.e1–381.e8.
- 1404 183. Hiwarkar P, Kosulin K, Cesaro S, Mikulska M, Styczynski J, Wynn R, Lion T. 2018.
1405 Management of adenovirus infection in patients after haematopoietic stem cell transplantation:
1406 State-of-the-art and real-life current approach: A position statement on behalf of the Infectious
1407 Diseases Working Party of the European Society of Blood and Marrow Transplantation. *Rev Med*
1408 *Virol* **28**:e1980.
- 1409











